ISSN 0037-9832

VOL 28 NO 7 JULY 1977

Journal of the Society of Cosmetic Chemists

Contents

Cosmetic Ingredients

Among our extensive range:

Allantoin
Bromochlorophen
Dihydroxyacetone
Eusolex®-UV-Filter
Insect – Repellent
Preservative
Oxynex-Antioxydan
Sorbitol liqu
Thioglycollic ac
Vitamin

Active ingredients for cosmetics

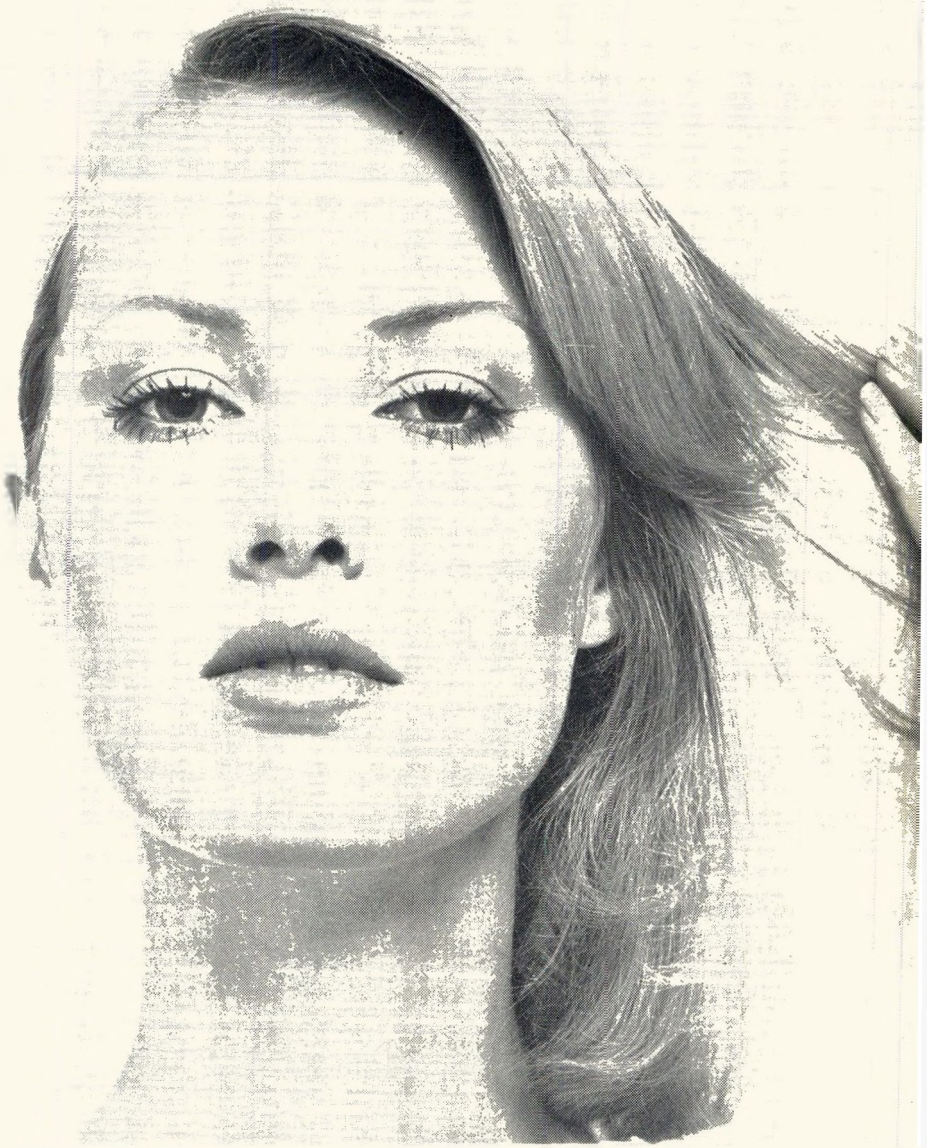
MERCK

THE QUALITY
YOU
CAN TRUST

Offers and technical information on request:

E. Merck, Darmstadt/Federal Republic of Germany

INDEX TO ADVERTISERS



The advantages of dealing with Amerchol are plain to see.

If you are in the market for lanolin derivatives and chemical specialties, Amerchol is the logical choice. We offer you a unique combination of great products and services. All backed up by our worldwide network of distributors, as well as our manufacturing plant in Vilvoorde, Belgium.

This new facility lets us give you the same Amerchol quality while providing you with better, faster service. For a wide range of products: Emulsifiers. Solubilizers. Emollients. Moisturizers. Lubricants. Humectants. U.V. Absorbers.

Amerchol products

ACETULAN® – Acetylated lanolin alcohols
AMERCHOL® – Multisterol extracts
AMERLATE® – Lanolin fatty acid derivatives
AMEROXOL® – Alkoxylated fatty alcohols
AMERSCREEN® – U.V. absorbers
GLUCAM™ – Alkoxylated glucose derivatives
MODULAN® – Acetylated lanolin
OHlan™ – Hydroxylated lanolin
POLYLAN® – Lanolin alcohol linoleate
SOLULAN® – Alkoxylated lanolin derivatives.

Get the best. Amerchol.

Amerchol®
a Unit of CPC International Inc
Amerchol Park, Edison, New Jersey 08817 USA

ANSTEAD

For technical information or samples in the United Kingdom contact
D.F. Anstead Ltd., Victoria House, Radford Way, Billericay, Essex CM12 0DE, England
Telephone Billericay (STD 02774) 53131
Telex 99410

Journal of the Society of Cosmetic Chemists

This edition is published for

THE SOCIETY OF COSMETIC CHEMISTS
OF GREAT BRITAIN

by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL
Hon. Editor: J. M. Blakeway
Roure Bertrand Dupont S.A. 55, Voie des Bans, 95100 Argenteu' ance)

VOL. 28 JULY 1977 No. 7

GENERAL NOTICES

Publication dates: The 'Journal of the Society of Cosmetic Chemists' is published on the 5th of each month.

Five issues for the Society of Cosmetic Chemists of Great Britain
56 Kingsway London WC2B 6DX.

Seven issues by the Society of Cosmetic Chemists
50 East 41 Street, New York, N.Y. 10017, U.S.A.

Issue No	*Publication Date*	*Country of Origin*
1	January	Great Britain
2	February	U.S.A.
3	March	U.S.A.
4	April	Great Britain
5	May	U.S.A.
6	June	Great Britain
7	July	Great Britain
8	August	U.S.A.
9	September	U.S.A.
10	October	Great Britain
11	November	U.S.A.
12	December	U.S.A.

Advertisements: All enquiries regarding advertisements in the British Editions of the Journal should be addressed to Blackwell Scientific Publications, Osney Mead, Oxford OX2 0EL.

Subscription: All members of the Society of Cosmetic Chemists of Great Britain receive one copy of each edition free. Further copies at non-member rates. Industrial and non-member subscribers: £40; overseas £48. If payments are made by bank transfer, all charges shall be at the remitter's expense.

Missing numbers: Journals are despatched at Printed Paper rate. Claims for missing numbers can be entertained only from subscribers in the country of origin of the particular issue, and must be made within 30 days from date of issue. Members and subscribers are urged to give notice of change of address to the Publications Offices.

Responsibility for statements published: The Society of Cosmetic Chemists of Great Britain and its Hon. Editor assume no responsibility for statements or opinions advanced by contributors to this Journal.

Lectures: The Society shall have the right of first publication of any lecture or address delivered before it, but does not undertake to publish any given matter.

Manuscripts: These should be in accordance with the '*Directions for the preparation of manuscripts*', copies of which are available from the Society of Cosmetic Chemists of Great Britain, 56 Kingsway, London WC2B 6DX.

SYNOPSES FOR CARD INDEXES

The following synopses can be cut out and mounted on 127 × 76 mm index cards for reference without mutilating the pages of the Journal.

The influence of perfumes on the sentitising potential of cosmetic bases I. A technique for evaluating sensitising potential: M. F. BRULOS, J. P. GUILLOT, M. C. MARTINI and J. COTTE. *Journal of the Society of Cosmetic Chemists* **28** 357–365 (1977)

Synopsis—A technique was developed for determining the sensitising potential of cosmetic products in the albino guinea-pig. It consists of the administration of Freund's complete adjuvant by intradermal injection and subsequent application of the test substance topically using an occlusive path. The technique is therefore particularly well suited for the testing of finished products.

The influence of perfumes on the sensitising potential of cosmetic bases II. The sensitising potential of perfumes and cosmetic bases: A. ROCHAS, J. P. GUILLOT, M. C. MARTINI and J. COTTE. *Journal of the Society of Cosmetic Chemists* **28** 367–375 (1977)

Synopsis—A sensitisation test was used to test a series of cosmetic formulations with and without the addition of perfumes. The reactions obtained were examined and compared with those caused by benzylideneacetone, a reference sensitiser, which was added to the excipients at the same concentration as the perfumes.

Safety evaluation of cosmetic raw materials: J. P. GUILLOT, M. C. MARTINI and J. Y. GIAUFFRET. *Journal of the Society of Cosmetic Chemists* **28** 377–393 (1977)

Synopsis—Tests were carried out for safety evaluation using the rabbit on twenty-six cosmetic components. The ocular and cutaneous tolerance was evaluated using official French methods with some additions. The results show that thirteen samples gave adverse reactions after repeated skin exposures; eight of them appeared to be significantly moer irritant—four isopropyl myristates and four oleyl alcohols each from different sources. Eleven were well tolerated when applied undiluted during 60 days and two gave uncertain results.

The mechanism of skin pigment production: P. A. RILEY. *Journal of the Society of Cosmetic Chemists* **28** 395–401 (1977)

Synopsis—The general metabolic pathways leading to the production of pheomelanins and eumelanins are outlined. This paper surveys the evidence that two types of oxidation by tyrosinase are involved, namely oxygen addition to monophenols (cresolase activity) and dehydrogenation of diphenols (catecholase activity). Highly reactive quinones are formed as intermediate metabolites and it is suggested that they are of importance as possible sources of perturbation of cell metabolism.

Enhancement of pigmentation: psoralens: RODNEY P. R. DAWBER. *Journal of the Society of Cosmetic Chemist* **28** 403–406 (1977)

Synopsis—Biological extracts of various common plants have been used in depigmenting conditions for many centuries to enhance pigmentation. Specific photodynamic chemicals have been extracted from such sources—psoralens. Synthesis of these substances in 1947 led to detailed laboratory and clinical investigation of their mode of action and effectiveness in increasing normal pigmentation and repigmenting vitiliginous skin. The theoretical fear that long-term psoralen and ultraviolet radiation treatment might induce skin tumours has not so far been realised in practice.

Journal of the Society of Cosmetic Chemists

VOL. 28 1977

Skin bleaching preparations: S. S. BLEEHEN. *Journal of the Society of Cosmetic Chemists.* **28** 407–412 (1977)

Synopsis—A brief review is given of the research for an effective and safe depigmenting compound and the screening of topically applied chemicals. The possible modes of action of these compounds in producing cutaneous depigmentation are discussed. The results of treatment using several skin bleaching preparations including a new formulation of hydroquinone and 4-isopropylcatechol cream in the therapy of various hypermelanotic disorders in man are stated.

J. Soc. Cosmet. Chem. **28** 357–365 (1977)

The influence of perfumes on the sensitising potential of cosmetic bases

I. A technique for evaluating sensitising potential

M. F. BRULOS*, J. P. GUILLOT†, M. C. MARTINI* *and* J. COTTE*

Received 4 January 1977

Synopsis

A technique was developed for determining the **sensitising potential** of **cosmetic** products in the albino **guinea-pig**. It consists of the administration of Freund's complete adjuvant by **intradermal injection** and subsequent application of the test substance topically using an occlusive patch. The technique is therefore particularly well suited for the testing of finished products.

INTRODUCTION

At both national and European levels legislation concerning cosmetic products is now being drawn up. French legislation already demands a range of animal tests relating to ocular and cutaneous irritation (1) but as yet official requirements do not include sensitisation tests.

The incidence of sensitisation reactions resulting from the use of cosmetic products is relatively low representing about four cases per million units sold (15).

The performance of sensitisation tests on healthy human subjects is forbidden in France – such tests pose delicate ethical problems – it is therefore pertinent to search for a simple test to perform on animals.

A review of the literature reveals that the techniques for such tests are diverse and numerous (6) their plethora being perhaps an indication of their limitations. All the test methods use a limited number of animals and rely upon increasing the sensitivity of the test animal in one or more of the following ways (7) (8): the use of high concentrations (20) (3). This is rarely suitable for cosmetic bases as tests are frequently carried out on the undiluted product; the special preparation of the treatment area (scarification or stripping) to accelerate penetration (18); the use of Freund's adjuvant (11) (12).

Frequently a route of administration is used which is incompatible with the texture or consistency of most cosmetic products (4) (11) (12) (9).

* Institut de Pharmacie Industrielle, Cosmétologie, 8 avenue Rockefeller, 69008 Lyon, France.

† Institut Français de Recherches et Essais Biologiques, Les Oncins, B.P. 109, 69210 L'Arbresle, France.

A test suitable for use with most cosmetic substances must meet the criteria outlined below.

Elimination of risks of parasitic irritation.
Detection of weak sensitisers.
Protocol design to suit finished products, i.e. sufficiently sensitive to function without increasing the concentration of the test product – topical rather than intradermal application of the test substance. Intradermally injected substances are sometimes absorbed with difficulty, particularly in the case of such cosmetic products as talcum powder and pigmented cosmetics, and may give false positive results since the dermal-epithelial barrier is bypassed.
Evaluation of reaction site by a simple but stringent test using only clearly positive macroscopic readings backed up by histological examinations.
Interpretation of response so that an 'all or nothing' result is obtained.

A bibliographic study of those factors influencing the induction of release of a hypersensitive reaction, coupled with a number of preliminary trials (2) has led to the development of a technique which meets the above criteria.

PRINCIPLE

Sensitisation in the guinea-pig is induced by intradermal injections of Freund's adjuvant and by topical applications of the test substance under occlusive dressings. After a rest period of 12 days a single challenge application of the test substance, again under an occlusive dressing, provokes the appearance of a sensitisation reaction.

The use of occlusive patches to increase the hydratation of the skin and permeability of the stratum corneum (10) plus the administration of Freund's complete adjuvant to maximize the immunological response gives the technique sufficient sensitivity to detect even weak allergens.

EXPERIMENTAL PROCEDURE

Animals

Albino Hartley guinea-pigs of both sexes are used. They weigh between 300 and 400 g.

ACCOMMODATION AND DIET

Two weeks before the start of the study, and for the 40 days duration of the study the animals are kept in an air conditioned animal house in cages of five measuring 600 × 540 × 315 mm. The cages have grilled bases to eliminate soiling with faeces, litter, etc. Each animal is fed 50 g of granules per day (granulés Cobaye U.A.R. N° 114). This diet is supplemented with carrots. Water is freely available.

SCREENING FOR PRIMARY IRRITATION

Before commencing the sensitisation study it is essential to check that the test substance does not cause primary irritation (application of test substance for 48 h under occlusive patch using six guinea-pigs).

All substances producing primary irritation are eliminated unless destined to be used in a diluted form. For these latter cases the minimum dilution which does not cause

irritation is determined and used for the challenge application. Induction is always performed using the undiluted test substance.

METHOD

The usual physico-chemical and bacteriological tests are first conducted on the test substances.*

The completion of the trial takes 6 weeks and uses twenty animals.

ELIMINATION OF ANIMALS SHOWING INDIVIDUAL IRRITATION REACTIONS

On day 0, 0·5 g or 0·5 ml of the test substance (or the dilution to be used for the challenge application) is applied to the back of the animal immediately behind the left scapulum. The test substance rests in place beneath an occlusive patch for 48 h. Animals are eliminated if any anomalies are revealed at the site after reading at 1, 7, 24 and 48 h following the removal of the patch.

INDUCTION

Induction consists of two intradermic injections of Freund's complete adjuvant† and ten applications of the test substance under occlusive patches. The first day of each week the animals are clipped in a region of the back just behind the right scapulum. On days 0 and 9 the guinea-pigs receive an intradermal injection of 0·1 ml of Freund's complete adjuvant diluted to 50% in sterile isotonic saline solution. The two injection sites should be as close together as possible at the centre of the clipped area.

On days 0, 2 and 4 (first week), days 7, 9 and 11 (second week), days 14, 16 and 18 (third week) and day 21, 0·5 g or 0·5 ml of the test substance is applied to the clipped test site of each animal.

The test substance is covered by an impermeable occlusive patch 22 mm in diameter. This is held in place by a microporous adhesive border 10 mm wide (Neodermotest). The entire patch is covered between treatment times by an elastic sleeve. The tenth patch is removed on day 23.

SUSPENSION OF TREATMENT

Treatment is stopped between days 23 and 35, i.e. for a total of 12 days.

CHALLENGE APPLICATION

On day 35 an area of the abdomen immediately above the left groin is clipped free of fur. At this site 0·5 g or 0·5 ml of test substance (or the chosen dilution) is applied under an occlusive patch for 48 h using the same system as already mentioned

A schematic summary of the procedure of the trial is given in *Figure 1.*

* In particular the peroxide value must be determined for fats and essential oils to ensure freshness.

† Biomérieux.

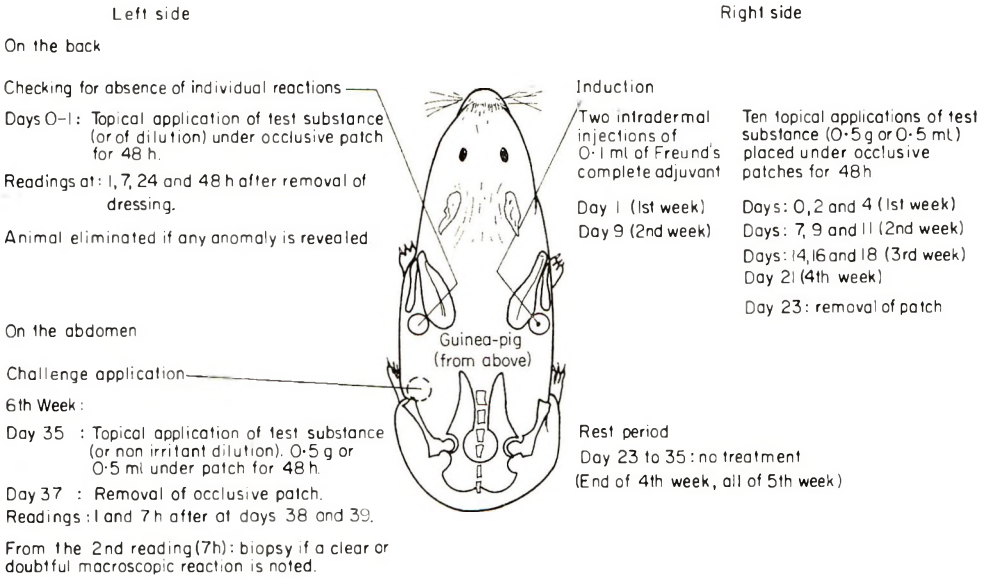


Figure 1.

INTERPRETATION OF RESULTS

EVALUATION OF THE REACTION

Macroscopic cutaneous examination

One, 7, 24 and 48 h after the removal of the occlusive dressing the intensity of the erythematous reaction and eschar formation are evaluated using the following scale:

no erythema	0
slight erythema (hardly visible)	1
erythema distinct	2
erythema moderate to severe	3
erythema severe (red/purple) with the formation of light eschars (profound lesions)	4

N.B. Any other anomaly occurring at the challenge site (eg. papules, vesicles, oedema exfoliation) should also be noted. The induction site must also be unspected in case of any focal reaction (reactivation of induction site). It is extremely important that all readings are performed under the same conditions (particularly the same conditions of lighting).

The results are interpreted by calculating the percentage of guinea-pigs sensitised. The animals counted as positive are those which, (a) present at least once in four readings a reaction scoring two or more, or (b) present focal reactions whatever the reaction observed at the challenge site, or (c) present vesicles.

Biopsies

About 6–7 h after removing the dressing samples of skin for histological examination are taken from the challenge sites of those animals showing distinct macroscopic

reactions. Samples are taken immediately after the readings which demonstrate these reactions.

It was thought that a macroscopic examination of the lymph glands or a comparative cytological examination of the lymph glands would give a confirmation of macroscopic results. Unfortunately Freund's complete adjuvant affects all lymph ganglions modifying their form, consistency and structure and causing the appearance of pyroniphilic cells even in those ganglia furthest from the induction site. A histological examination of the skin sites was therefore found to be more fruitful.

Histological examination

After fixing fragments of skin in Bouin's solution they are embedded in paraffin, sectioned at five microns and stained with haematoxylin and eosin.

When the reaction is allergic in nature the sample has the aspect of an experimental eczema. The anomalies revealed are therefore: inflammatory peri-capillary infiltration of lymphoid cells with active congestion of vessels of the superficial dermis; exocytosis of lymphocytes towards the epidermis and across this towards the stratum corneum; intra-epidermal oedema (spongiosis) sometimes forming sub-corneal collections of liquid leading to vesicles; discontinuity of the stratum granulosum; surface appearance of nuclei in cells of the corneal layer (parakeratosis) between which some serous exudation is found.

Later on an increase in mitotic activity of the cells of the stratum basale may occur with an increase in the number of keratinocytes of the stratum Malpighi (hyperacan-

When primary irritation only is noted the alterations are different, namely: the corneal layer forms pleated folds; sub-corneal eosinophilic necrosis is seen which may or may not be profound. At worst detached bubbles may develop; capillary congestion of superficial dermis with polymorphic infiltration, always containing a large number of granulocytes; intra-epidermal exocytosis formed only of granulocytes.

However, the reaction phenomena, parakeratosis, hyperacanthosis and papillomatosis are identical to those seen with allergic reactions.

Furthermore, when the biopsy is conducted late, or when intolerance is not very marked a histological examination does not allow a definitive distinction between these two mechanisms to be made. Paradoxically it can also be difficult to distinguish between these mechanisms when intolerance is such that it results in eschar formation particularly since some substances provoke both caustic and sensitisation reactions. One should not ask too much of the classical histological examination, it has its limitations and should be regarded as one of the aids when formulating a general conclusion.

Expression of results

After the macroscopic cutaneous examination and histological examination have been conducted 'blind', the results are evaluated as shown below.

The result is positive if one or more animals show distinct macroscopic reactions confirmed histologically as sensitisation reactions.

The result is negative if no animal shows a distinct macroscopic reaction or if the histology does not confirm the macroscopic observation.

The result is doubtful if a distinct macroscopic reaction is noted but the histological examination is unable to determine its origin.

Table I.

Data at hand	Test substance: Chemical name	Conc.	Vehicle	Macroscopic examination: % animals reacting	Mean of erythema score	Histological examination	Result
Experimentally a strong sensitiser for both man and the guinea-pig Has caused numerous sensitisations at concentrations of 2% (5)	Paraphenylene-diamine	2%	Ethanol (70°)	100	2·16	2/3 showed allergic type inflammatory reactions with intense spongiosis and massive lymphocyte exocytosis. In one there was necrosis, with erosion, weeping and a squamous crust	+
		0·5%	Ethanol (70°)	93·7	2·20	5/6 showed inflammatory reactions which were clearly allergic with spongiosis	+
Experimentally a weak sensitiser for man and the guinea-pig	Benzocaine	2%	Sterile neutral olive oil	38·8 38·8	1·47	1/2 showed a moderate allergic reaction. The epidermis was slightly thickened. Serous parakeratosis and lymphocyte exocytosis	+
Has caused sensitisation at concentrations of 1% (13)		0·5%	Sterile neutral olive oil	29·4	1·36	1/3 showed a clearly allergic response with spongiosis and sub-normal vesicles	+
No experimentally positive results in animal. Has caused sensitisation in man at concentrations of 5% (14)	Butyl parahydroxy-benzoate	5%	Sterile neutral olive oil	29·4	1·70	2/6 showed pathological aspects. The worst showed spongiosis, weeping, squamous crust and moist lymphocyte infiltration. Aspect clearly allergic	+
No sensitising reactions observed in man in use	Shampoo	30%*	Water	0	0·15	3/3 showed no allergic response	—

Experimentally a class III sensitiser in the guinea-pig and class IV in man (10) (11). Has caused many sensitisations (5)	Hydroquinone monobenzyl ether	5%	Ethanol (70°)	47	1·48	No pathological aspect was noted (3/3)	–
Experimentally sensitising in man at 20% (17)	Dihydrocoumarine	1%	Ethanol (70°)	57.1	1·82	1/3 gave a pathological picture of allergic type with moist parakeratosis, exocytosis and slight spongiosis. Allergic type	+
Experimentally sensitising in man at 4% (17)	Citral	1%	Ethanol (70°)	61·1	1·88	3/7 showed an aspect clearly pathological with one distinctly allergic type with spongiosis	+
Experimentally a strong sensitiser in man at 2% (17)	Benzylidene acetone	1%	Ethanol (70°)	66·6	1·81	3/6 showed lesions of allergic type with intense spongiosis, exocytosis and weeping	+
		1%	Aqueous lotion	70·5	2·15	2/3 showed reactions of allergic type. One was a major reaction with necrosis and erosion similar to an intolerance of mixed type (caustic and allergic)	+
		1%	Emulsion water in oil	93·7	1·97	3/3 showed a pathological aspect. In one the inflammatory process was clearly allergic with spongiosis, lymphocyte exocytosis and intense parakeratosis	+
		1%	Emulsion oil in water	100	2·39	2/3 showed very slight surface parakeratosis with neither spongiosis nor exocytosis. Aspect pathological, but difficult to classify	Doubtful

* The induction (10 applications was made at 100%).

Reliability

Using the technique described above several compounds known to be sensitisers were tested. The results are given in *Table I.*

DISCUSSION

Of the twelve preparations tested (seven different substances) ten gave good correlation between the macroscopic and histological results.

The 5% hydroquinone monobenzyl ether made up in ethanol (70°) and the benzylideneacetone in an oil/water emulsion gave questionable results, as the macroscopic observations were not confirmed histologically. However, instead of being taken 7 h after the removal of the occlusive dressing, samples were taken 48 h after removal (i.e. 96 h after application of test substance). When one examines the macroscopic development of cutaneous reactions one notes a diminution in the intensity of the erythema between 24 and 48 h after the removal of the occlusive dressing. Perhaps in this case the biopsies were conducted too late.

For benzylideneacetone the histological examination gave rather indeterminate results for similar reasons. Other studies conducted with other oil/water emulsions containing benzylideneacetone have demonstrated reactions which are of primary irritation in nature.

It is therefore important to perform skin biopsies no later than 7 h after the removal of the patch to reduce the incidence of false negative results.

Precautions were taken to avoid interference due to irritation. However, even in healthy areas, the skin of sensitised subjects is more susceptible to irritation than the skin of normal subjects. Furthermore the use of Freund's adjuvant may lead to the appearance of non-specific reactions.

It must also be remembered that the margin between the maximum non-irritant concentration and the minimum necessary to cause a sensitisation reaction is much smaller in the guinea-pig than in man (16). This is why it seems necessary to consider only those macroscopic reactions scoring two or more which also reduces the need to conduct a histological examination.

We abandoned the Magnusson and Kligman system for the expression of results.

Cosmetic products ready for commercialization are unlikely to contain strong sensitisers since known sensitisers are eliminated from their composition. Unlike a pharmaceutical product where the therapeutic activity may be great enough to permit the use of a component with doubtful sensitising potential, uncertainty is never acceptable for a cosmetic product. Thus it is important to be clear about the expression of results during the animal test (the more so since extrapolation to man entails many approximations).

It is possible to use this technique for detecting the sensitizing potential of cosmetic bases (aqueous or oil solutions, and water/oil or oil/water emulsions) as well as the potential of the perfume content or the combined mixture (19).

CONCLUSION

The method described here gave good results for the detection of weak sensitizers. Since this test avoids the use of an increase in concentration to maximize the reaction nor

does it make use of intradermal injections as the route of administration it seems particularly well adapted to the testing of finished products whatever their form.

ACKNOWLEDGMENT

The histological examinations were performed and interpreted by Dr J. Guilaine.

REFERENCES

1 Méthodes officielles d'analyse des cosmétiques et produits de beauté. Annexes I, II, III. Arrêté du 5 avril 1971. *Journal Officiel de la République française,* 21 April 3862 (1971).
2 Brulos, M. F. *Mise au point d'une méthode d'evaluation du pouvoir sensibilisant des produits cosmétiques* (test prophétique). *Thèse de doctorat* de l'Université de Pharmacie. Lyon 14 September 1976.
3 Buehler, E. V. Delayed contact hypersensitivity in the guinea-pig. *Arch Dermatol.* **91** 171 (1965).
4 Draize, J. H. Dermal Toxicity. Dans: *Appraisal of the safety of chemicals in foods, drugs and cosmetics,* 46–59 (1969).
5 Fischer, A. A. *Contact dermatitis.* Lea et Febiger, 2nd edition. 1973 Philadelphia.
6 Hardy, J. Allergy, hypersensitivity and cosmetics. *J. Soc. Cosmet. Chem.* **24** 423–468 (1973).
7 Hendeison, C. R. and Riley, E. C. Certain statistical considerations in patch testing. *J. Invest. Derm.* **6** 226, 230 (1945).
8 Knudsen, L. Note on statistical probabilities of finding hypersensitive subjects in random samples. *J. Invest. Dermatol.* **6** 231–232 (1945).
9 Landsteiner, K. and Jacobs, J. Studies on the sensitisation of animals with simple chemical compounds. *J. Exptl. Med.* **61** 643–656 (1935).
10 Magnusson, B. and Hersle, K. Patch test methods III : Influence of adhesive tape on test response.
11 Magnusson, B. and Kligman, A. The identification of contact allergens by animal assay. The maximisation test. *J. Invest. Dermatol.* **52** 268–276 (1969).
12 Magnusson, B. and Kligman, A. *Allergic contact dermatitis in the guinea-pig. Identification of contact allergens.* Charles C. Thomas, Springfield. 1970.
13 Marzulli, F. N., Carson, T.R. and Maibach, H. I. Delayed contact hypersensitivity studies in man and animals. *Proc. Joint. Confer. Cosmt. Sci.* Washington D.C. p. 107–122 (1968).
14 Marzulli, F. N. and Maibach, H. I. Antimicrobials experimental contact sensitisation in man. *J. Soc. Cosmet. Chem.* **24** 385–421 (1973).
15 Masters, E. J. Allergies to cosmetic products. *N. Y. State. J. Med.* **60** 1934–1941 (1960).
16 Nilzen, A. Some aspects of epidermal testing in guinea-pig sensitised and not sensitised to 2.4. dinitrochlorobenzene. *Acta Dermato Venereol.* **32** supplement 29 231–239 (1952).
17 Opdyke, D. J. Monographs on Fragrant Raw Materials. *Foods and Cosmet. Toxicology.* **11** 95–115, 477–495, 855–876, 1011–1081, (1973); **12** 385–405, 517–537, 703–736 (1974); **13** 91–112, 449–458, 545–554 (1975).
18 Krafr, E. R., Hoch, S. G., Quisno, R. A. and Newcomb, E. A. Evaluating the safety of cosmetics by human patch-test methods. Symposium 'Peau et environnement' *J. Soc. Cosmet. Chem.* **23** 383 (1972).
19 Rochas, A., Guillot, J. P., Martini, M. C. and Cotte, J. Contribution à l'étude de l'influence des parfums sur le pouvoir sensibilisant de bases cosmétiques. 2ème partie: role du parfum sur le pouvoir sensibilisant de bases cosmétiques. *J. Soc. Cosmet. Chem.* This issue.
20 Voss, J. G. Skin sensitisation by mercaptans of low molecular weight. *J. Invest. Derm.* **31** 273 (1958).

J. Soc. Cosmet. Chem. **28** 367–375 (1977)

The influence of perfumes on the sensitising potential of cosmetic bases

II. The sensitising potential of perfumes and cosmetic bases

A. ROCHAS*, J. P. GUILLOT†, M. C. MARTINI‡ *and* J. COTTE‡

Received 4 January 1977

Synopsis

A sensitisation test was used to test a series of **cosmetic** formulations with and without the addition of **perfumes**. The reactions obtained were examined and compared with those caused by **benzylideneacetone**, a reference sensitiser, which was added to the excipients at the same concentration as the perfumes.

INTRODUCTION

The development of an animal test technique for evaluating sensitising potential in the guinea-pig was described in Part I of this paper. The test was devised for use with finished cosmetic preparations for which the Magnusson–Kligman test is not entirely suitable. This section of the paper considers the influence of the addition of several perfumes to typical cosmetic bases. A test substance which has a known allergenic activity was used for comparative purposes.

OBJECTIVE

Under certain conditions and in certain individuals most substances are potentially allergenic. The chances of any substance being allergenic increases with the complexity of the preparation. With this in mind the research had two aims: to test the eventual sensitising potential of several cosmetic preparations; to determine whether the addition of a perfume (of which the sensitising potential was inferior to threshold detection) to a non-sensitising base was likely to lead to a finished product which was a sensitiser and to examine if this phenomenon was related to the cosmetic form used.

* Laboratoire de Physiologie B, 8 ave. Rockefeller, 69008 Lyon, France.

† Institut Français de Recherches et Essais Biologiques, Centre de Lyon, Les Oncins, 69210 L'Arbresle, France.

‡ Institut de Pharmacie Industrielle, Cosmétologie, 8 ave. Rockefeller, 69008 Lyon, France.

PROCEDURE

TEST SUBSTANCES

Cosmetic bases

The following bases were used: a water-based lotion; three oil in water emulsions which differed in the proportion of the oil phase and in the type of fats used, and in the proportion of surfactant emulsifier; two water in oil emulsions differing in the mixture of fats used, in the emulsifier used, and in the preservative.

All the raw materials used and the finished products were submitted to physico-chemical analyses and bacteriological control.

Perfumes (see Appendix)

The following were incorporated at 1% into the cosmetic bases: a reference substance: benzylideacetone (3); an aromatic base; perfume preparations containing 34%, 54%, 67% or 74% of the aromatic base mentioned above.

METHOD

Details of the methods used can be found in the first part of this paper (1). An outline of the principle of the method is as follows:

Sensitisation in the guinea-pig is induced by intradermal injections of Freund's adjuvant and by topical applications of test substances under occlusive dressings. After a rest period of 12 days a single challenge application of the test substance, again under an occlusive dressing, provokes the appearance of a sensitisation reaction.

Readings were conducted immediately after the removal of the patch in order to eliminate errors due to subsequent scratching, etc. and then, one hour, 24 h and 48 h after removal of the patch.

The last reading at 48 h was accompanied if necessary by a skin biopsy for eventual histological examination. It is however, preferable to perform the biopsy 7 h after the removal of the patch, as explained in Part I. The erythema was scored from 0 to 4 and any other anomalies (papules, vesicles, exfoliations) or a reactivation of the induction site were noted.

The number of animals showing an evident reaction (equal to or greater than 2) at any of the readings was calculated and the mean erythema value for the whole group was also determined.

The aim of the histological examination was to determine the allergic characteristics of the reaction. In fact when the macroscopic examination was positive, a histological examination was also conducted. When this examination was positive, sensitization was confirmed. If this examination was negative, for instance, if the reaction noted was of primary irritation, then the overall result was said to be negative. When the macroscopic examination was negative, the histological examination was not conducted.

The test was called 'doubtful' when the macroscopic examination was positive and the histological examination was unable to determine the type of reaction present.

The interpretation of the results can therefore be summarized in *Table I.*

Table I. Classification of observations

Macroscopic examination	Histological examination	Results
+	+ (allergy)	+
+	− (orthoergy)	−
+	doubtful	doubtful
−	not conducted	−

During this work which was conducted on groups of fifteen animals the histological examination was systematically conducted on all animals showing a positive macroscopic reaction and on some (3 to 6) which did not show a macroscopically positive reaction or which showed a doubtful reaction.

The differences between the procedure described here and the method suggested in Part I of the paper result from the fact that our studies allowed us to improve the procedure of the experiment. Therefore, groups of fifteen guinea-pigs were used throughout although larger groups would give a greater precision. The primary cutaneous irritation score of bases and perfume-base mixtures was first evaluated using the rabbit following the method in the 'Journal Officiel Français'. The same concentration was confirmed as non-irritant with the guinea-pigs used in the tests. Biopsies for histological examinations were taken 48 h after removal of the patch covering the challenge application and not after 7 h which is preferable and which is suggested in the final method.

RESULTS

The cosmetic bases on their own (*Table II*) and the base-perfume mixtures all gave negative results when tested for primary cutaneous irritation in the rabbit.

For the same preparations, no evidence was obained of individual irritation reactions in the guinea-pig. The erythema observed after the removal of the first patch was always equal to one or less.

On the contrary, when 1% benzylideneacetone was added to the bases, primary cutaneous irritation reactions were noted in all animals. In order to demonstrate the sensitising potential of benzylideneacetone without the problems of irritation it would be necessary to conduct a challenge reaction with a diluted solution.

The intensity of the erythema observed at the end of the sensitisation test was always clearly greater than that observed after the first application even though, as will be seen later, irritation phenomena were dominant.

Details of the results of the sensitisation test are shown in the *Tables II* to *V*. They show the reactions due to the bases on their own, the bases with the addition of the perfumes and details of the reactions obtained using benzylideneacetone. In *Table II* only the oil in water preparation, C, exhibited a potential sensitizing activity. Cream B, the allergic reaction was extremely slight and can be considered as negative.

Whatever the medium, the addition of benzylideneacetone resulted in reactions in nearly all subjects. There were some cases of allergic reactions. In other cases these reactions were masked by important orthoergic reactions which were evident in all animals from the first application.

The intensity and character of the reaction does not seem to differ with the type of emulsion.

Table II. Results of sensitisation tests on unperfumed cosmetic means

Bases		Number of animals showing reactions*	Mean of erythemas per group	Macroscopic examination (inter-pretation)	Histological examination			
					Number of animals examined	Reactions	Inter pretation	Conclusion
Lotion	— A —	1/10†	0·13	+	6	No reaction noted	−	−
Cream O/W	— A —	2/9	0·63	+	3	Moderate primary irritation in two animals	−	−
Cream O/W	— B —	1/13	0·60	+	6	Minor allergic response in one animal	−	−
Cream O/W	— C —	5/13	0·75	+	6	Major allergy in one animal	+	+
Cream W/O	— D —	0/14	0·33	−	6	No reaction noted	−	−
Cream W/O	— E —	0/11	0·56	−	6	No reaction noted	−	−
Reference Benzylideneacetone ethanol 70°		7/11	1·81	+	6	Major allergy in three animals	+	+

* i.e. with an erythema score of two (evident erythema) at one of the three readings, or a focal reaction, or presence of vesicles.

† The figures correspond to the number of survivors.

Table III. Influence of the addition of a known sensitiser (1% benzylidenacetone) to cosmetic bases

Bases		Number of animals showing reactions	Mean erythemas per group	Macroscopic examination (inter-pretation)	Histological examination			
					Number of animals examined	Reactions	Inter-pretation	Conclusion
Lotion	— A —	7/8	2·34	+	3	Mixed allergic and caustic reaction	+	+
Cream O/W	— A′ —	not performed						
Cream O/W	— B —	9/9	2·22	+	4	Moderate orthoergy	–	–
Cream O/W	— C —	5/5	2·47	+	3	Moderate orthoergy	–	–
Cream W/O	— D —	10/10	2·67	+	4	Marked orthoergy	–	–
Cream W/O	— E —	7/7	2·24	+	3	Allergy + orthoergy	+	+

Table IV. Evaluation of the sensitising potential for the perfume base no. 602 127, tested at 1% in ethyl alcohol 70% v/v

Product	Number of animals showing reactions	Mean of erythemas per group	Macroscopic examination (inter-pretation)	Histological examination			
				Number of animals examined	Reactions	Inter-pretation	Conclusion
No. 602 127 (1% in 70% v/v ethyl alcohol)	0/12	0·47	–	6	No reaction noted	–	–

Table V. Influence of addition of perfumes to cosmetic bases

Test substances	Number of animals showing reactions	Mean of erythemas per group	Macroscopic examination (inter-pretation)	Histological examination			
				Number of animals examined	Reactions	Inter-pretation	Conclusion
4.1/Lotion — A —							
Lotion only	1/10	0·13	–	6	No reaction noted	–	–
Lotion + perfume No. 602 123	0/12	0·02	–	3	No reaction noted	–	–
4.2/Creams O/W							
Cream O/W — A —	2/9	0·63	+	3	Moderate orthoergy two animals	–	–
+ perfume 602 127	0/12	0·27	–	3	Normal	–	–
+ perfume 602 125	1/11	0·25	+	3	Normal	–	–
Cream O/W — B —	1/13	0·60	+	6	Slight allergy? response (one animal)	–	–
+ perfume 602 127	2/12	0·27	+	3	Normal	–	–
+ perfume 602 124	2/13	0·38	+	3	Congested dermis (two animals)	–	–
Cream O/W — C —	5/13	0·75	+	6	Major allergy (one animal)	+	+
+ perfume 602 127	0/11	0·15	–	3	Normal	–	–
+ perfume 602 123	0/10	0·10	–	3	Desquamation in two animals	–	–
4.3/Creams W/O							
Cream W/O — D —	0/14	0·33	–	6	No reaction noted	–	–
+ perfume 602 127	1/13	0·38	+	3	No reaction noted	–	–
+ perfume 602 125	1/14	0·28	+	3	No reaction noted	–	–
Cream W/O — E —	0/11	0·56	–	6	No reaction noted	–	–
+ perfume 602 127	3/14	0·62	–	3	No reaction noted	–	–
+ perfume 602 124	2/12	0·24	+	3	One doubtful reaction	–	–

DISCUSSION

The results of these experiments with six excipients which were used as bases for four different aromatic components show that whatever the excipient used the number of animals showing reactions was always very small. In addition, the mean erythema scores were comparable and always inferior to 1 (1 = erythema barely visible).

Only one result seemed difficult to interpret – cream C: test positive with perfume and negative without perfume. It seems necessary to conduct a new series of tests for this cream.

The cutaneous tolerance showed little or no modification due to the perfumes (basic composition or elaborated compositions) with relation to the excipient used.

It should be emphasized however, that whenever a possible allergic type reaction was found for a base, or a perfume, or a mixture of the two this conclusion was based, in the majority of cases, on the effects found in one animal only. In addition, in all cases where there was an increase in the reaction score the mean of the erythema for the whole group always remained well below 1 whereas a test substance which is clearly irritant or sensitising is normally around 2·5 (erythema clearly visible).

CONCLUSIONS

This test seems sufficiently sensitive to demonstrate even slight cutaneous intolerance of the allergic type. Although only a limited number of perfumes were tested the results encourage the conclusion that there was no indication of producing a sensitising preparation from a base and a perfume which separately are non sensitisers.

ACKNOWLEDGMENTS

This study was instigated and supported by 'Les Laboratoires Vichy' and 'La Société Firmenich'. We also thank Dr J. Guilaine, Pathologique Hôpital Beaujon, Paris, who carried out the histological examinations.

RERERENCES

1 Brulos, M. F., Guillot, J. P., Martini, M. C. and Cotte, J. The Influence of perfumes on the sensitising potential of cosmetic bases. I: A technique for evaluating sensitising potential. *J. Soc. Cosmet.* **28** 357 (1977).

2 Magnusson, B. and Kligman, A. The identification of contact allergen by animal essay. The guinea-pig maximisation test. *J. Invest. Dermatol.* **52** 268–276 (1969).

3 Opdyke, D. L. J. Monographs on fragrance materials. *Food Cosmet. Toxicol.* **11** 1021 (1973).

APPENDIX I

COSMETIC BASES

EMULSION A

fat phase: 37% of a base consisting essentially of hydrogenated poly-butylene.
emulsifier: polyoxyethylene sorbitol monostearate + glycerol monostearate.
preservatives: a mixture of parahydroxybenzoic acid esters.

EMULSION B

fat phase: 40% of a base consisting of hydrogenated poly-isobutylene with the addition of a starch derivative.
emulsifier: polyoxyethylene sorbitol monostearate + glycerol monostearate.
preservative: a mixture of parahydroxybenzoic acid esters.

EMULSION C

fat phase: approximately 50% of a base consisting essentially of a mixture of synthetic esters (Pur Cellin) and mineral oil.
emulsifier: polyoxyethylene sorbitol monostearate + glycerol monostearate.
preservative: a mixture of parahydrobenzoic acid esters.

EMULSION D

fat phase: approximately 50% of a blend of lanolin derivatives, mineral oil and petroleum jelly.
emulsifier: Magnesium lanolate.
preservative: trichlorodiphenylether, imidazoline urea.

EMULSION E

fat phase: approximately 30% of a blend of fatty acid esters and mineral oil.
emulsifier: glycerol esters of fatty acids.
preservative: blend of parahydroxybenzoic acid esters.

LOTION A

This formula containing no alcohol is based on 40 % of floral waters, glycerine and demineralized water.
preservatives : blend of parahydroxybenzoic acid esters.
Colours F.D.C. : C.I. 42090
C.I. 14700

Both perfumed and unperfumed emulsions were subjected to microbiological control tests which gave no growth in all cases.

APPENDIX II

COMPOSITION OF THE PERFUMES USED IN THE TEST

Base composition (602 127)

Benzyl acetate	65
Styralyll acetate	5
Distilled Bergamot	38
Dipropylene glycol	212
Isoeugenol	7
Linalol	47
Musc indanone	53
Phenyl ethyl alcohol	67
Diethyl phthalate	203
Benzyl salicylate	200
Ylang Ylang	103
	1000

Formulae N° 602 123 to N° 602 126 have been prepared using N° 602 127 as a base:

602 123 : Perfume with a chypre citrus floral note, slightly woody, with lavender and amber
Base 602 127 represents 34% of this fragrance.

602 124 : Floral powdery odour with a strong hyacinth base.
Base 602 127 represents 54% of this perfume.

602 125 : Strong violet floral note with a powdery base and having slight green undertones.
Base 602 127 represents 67% of this perfume.

602 126 : a classic floral perfume, slightly spicy, with green hyacinth side notes.
Base 602 127 represents 74% of this perfume.

J. Soc. Cosmet. Chem. **28** 377–393 (1977)

Safety evaluation of cosmetic raw materials

J. P. GUILLOT*, M. C. MARTINI† *and* J. Y. GIAUFFRET‡

Presented at the XIth European Week of Dermocosmetology on 21 January 1977 at Lyon, France.

Synopsis

Tests were carried out for **safety evaluation** using the rabbit on twenty-six **cosmetic** components. The **ocular** and **cutaneous** tolerance was evaluated using official French methods with some additions. The results show that thirteen samples gave adverse reactions after repeated skin exposures; eight of them appeared to be significantly more irritant—four isopropyl myristates and four oleyl alcohols each from different sources. Eleven were well tolerated when applied undiluted during 60 days and two gave uncertain results.

INTRODUCTION

This work represents the first stage of a more ambitious programme, as it is proposed to test several series of products of various chemical compositions which form the basis of the majority of cosmetic formulations. The aim is three-fold: to allow the formulation chemists to know the tolerance to different categories of raw materials; to try to understand the origin of phenomena observed in tolerance tests of finished products; to establish 'skeleton' formulae for each type of product (milks, creams o/w and w/o etc.) composed of tested raw materials that have been qualified as non-irritant and to study in parallel the finished products based on the corresponding skeletons.

It is believed these results are significant because, carried out by the same personnel, they permit reliable comparisons.

Ocular and cutaneous tolerance to raw materials should be an essential consideration in the formulation of products intended for local use, whether they be cosmetic or pharmaceutical. Paradoxically, it would appear that this is a field which has not been extensively studied (10) (17). Often the formulator relies on information gathered over the years, information which is often falsified by the presence of other materials in the formula. In other cases, certain products are accepted as favourable without taking into account the fact that impurities can exist. In short, the rare experiments related are often fragmentary and unmethodical: they concern only a small percentage of raw materials and seldom serve to enlighten the users.

* Institut Français de Recherches et Essais Biologiques. Les Oncin 69210—L'Arbresle, France.
† Institut de Pharmacie Industrielle, 8 av. Rockefeller 69008—Lyon, France.
‡ R. & D. Lancaster (Beecham Products), 7 av. d'Ostende—Monte-Carlo, Monaco.

Toxicologists are primarly concerned by the allergic type intolerance, especially that to lanolin (1) (2) (3) (5) (6) (8) (9) (16) (20) (29). Also studied were the cetyl and stearyl alcohols (9) (12) (31), isopropyl alcohol (21), propylene glycol (14), stearic acid (24) (6), waxes (5) (30), propane diol (24) (6), vaseline and paraffin (30) (7) (31).

In fact, experiments on animals and humans are intended to show all the phenomena of ocular and cutaneous intolerance whether they be irritation or sensitisation (13) (19) (26) (28).

A recent study (25) has been carried out to examine the percutaneous absorption of five ^{14}C- labelled synthetic oils and an emulsion. The aim was to try to establish a relationship between the degree of penetration of the product and the observed intolerance. Perhaps it is here that an interesting start can be found to the problem of tolerance to natural and synthetic fatty materials.

EXPERIMENTAL

The test substances consisted of twenty-six samples chosen from those most used in cosmetics. Liquid products were tried first because of their ease of application.

Whenever possible, samples from several suppliers were tested. It was however impossible within the constraints of the investigation to test several lots from the same supplier.

The materials studied are given in *Table I.*

Table I. Raw materials tested and sample reference numbers

Squalane and substitute
squalane = substance n° /1/
hydrogenated polyisobutene = n° /2/
Triglycerides
Caprylic/capric triglycerides = /3/, /4/, /5/
Synthetic triglyceride = /6/
Esters
Isopropyl myristate = /7/, /8/, /9/, /10/
Isopropyl palmitate = /11/, /12/, /13/, /14/
Decyl oleate = /15/ & Isodecyl oleate = /16/
Octyl palmitate = /17/
Octyl stearate = /18/
Ceto-stearyl octoate = /19/
Stearyl heptoate = /20/
Arachidyl propionate ester = /21/
Oleyl alcohol = /22/, /23/, /24/, /25/
Polyoxyethylene sorbitan stearate = /26/

Each substance was applied both pure and in aqueous dispersion at 10–15%. For this it was necessary to obtain a simple suspension with an emulsifier and preservative to give a minimum stability, using the following formula:

Product 10–15%
Polyoxyethylene sorbitan stearate 3%
Preservative 0·2%
Water to 100%

TEST CONDITIONS

The experimental conditions were rigorously standardized: A physico-chemical analysis was carried out on each sample followed by gas chromatography and a determination of peroxide index to show any chemical deterioration or the presence of impurities.

The safety evaluation was conducted using the following tests:

Determination of the ocular irritation index (O.I.I.)
Determination of primary cutaneous irritation index after one occlusive application (P.I.I.)
Determination of cumulative cutaneous irritation index after repeated exposures (C.I.I.).

The test procedure is described in the 'Journal Officiel de la République Française' of 21.4.71 and 5.6.73 (15) but the following additions or modifications have been made;

Ocular irritation test:

Reading after 1 h, in addition to those of 24 h, 2d, 3d, 4d and 7d;
Photomotor reflex study;
Use of strips of sterile paper impregnated with fluorescein to help to demonstrate the presence of corneal opacification and to evaluate the extent of surface attack;
A qualitative evaluation of any ulceration or granulation;
Use of an ophthalmoscope and a retinograph;
Interpretation of the results using an evaluation scale from 0 to 100.

Primary cutaneous irritation test:

Use of occlusive patches 'Neodermotest';
Fixing of the patches using absorbent gauze held in place by adhesive tape;
Housing in individual cages (600 × 540 × 315 mm);
Modification of the evaluation scoring: non irritant, less than 0·5, slightly irritant 0·5 to 2 (cf. Journal Officiel non irritant=0 and slightly irritant from 0 to 2).

Cumulative cutaneous irritation test:

Reduction of the length of treatment: from 3 months to 8 weeks;
Wiping off the excess of substance with gauze;
Daily readings expressed as a weekly average;
Qualitative evaluation of thickening and drying of the skin;
After 8 weeks of treatment systematic histological examination of two samples of skin;
A study of recovery from cutaneous injuries, by stopping application for 7 days and examining the skin after this rest;
Challenge assay to determine whether the reactions observed have irritant or allergic origin.

RESULTS

Chemical study

In checking through specifications and analytical records, one must consider that the materials correspond to those regularly used in the industry. A qualitative selection was purposely not made as it was desirable to study those readily available on the market.

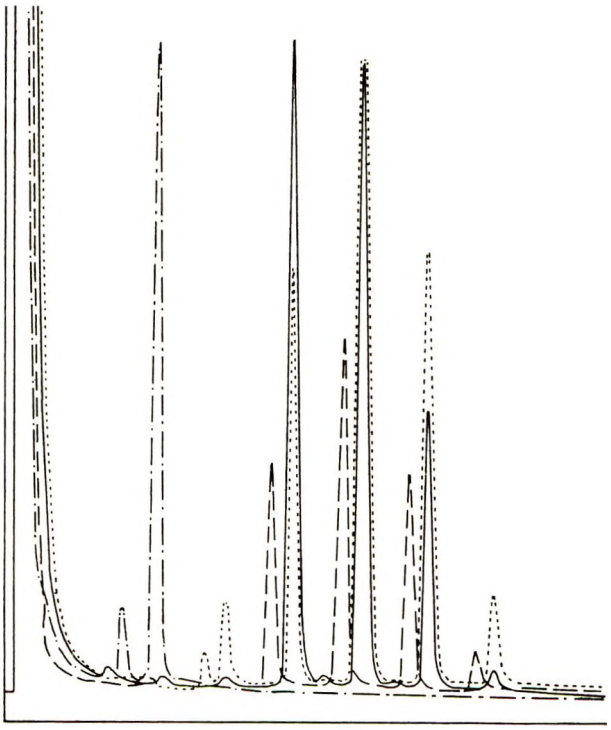


Figure 1. Comparative gas-liquid chromatograms of the triglycerides. Temperature programme: 240°–300°C. at 5°C./min on 2·5% SE 30, 80/100 mesh chromosorb W AW DMCS. Solvent: acetone. Temp. Inj.: 320°C. Col.: 1 min at 240°C. ——— = /3/; - - - - - - = /4/; ..... = /5/; —·—·— = /6/.

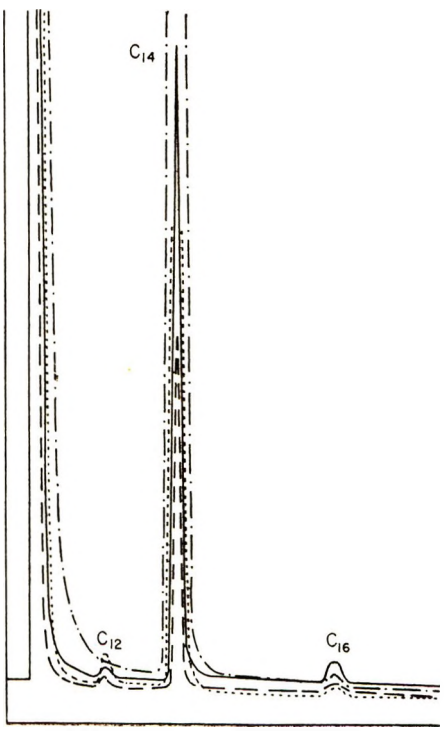


Figure 2. Comparative gas-liquid chromatograms of the isopropyl myristates. Temperature: 170°C. on 10% DEGS, 60/80 mesh chromosorb W HMDS. Temp. Inj.: 300°C. Col.: 2 m×¼ in. (glass). ——— = /7/; - - - - - - = /8/; ..... = /9/; —·—·— = /10/.

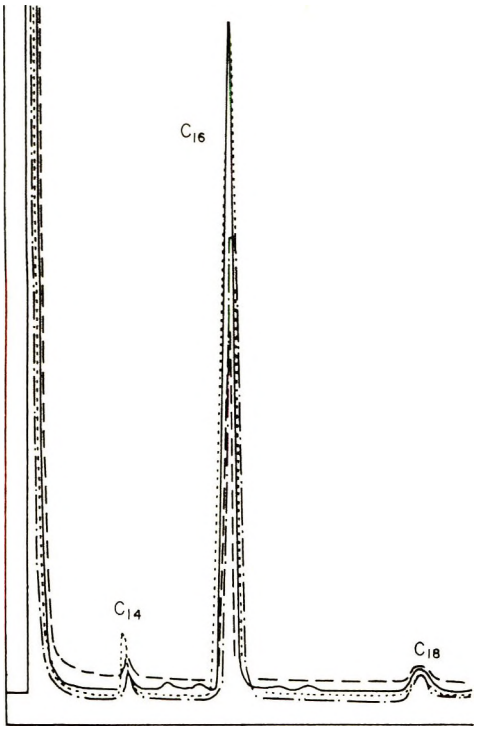


Figure 3. Comparative gas-liquid chromatograms of the isopropyl palmitates. Temperature: 180°C. on 10% DEGS, 60/80 mesh chromosorb W HMDS. Temp. Inj.: 320°C. Col.: 2 m × ⅛ in. (glass). ——— = /11/; - - - - - - = /12/; ..... = /13/; —·—·— = /14/.

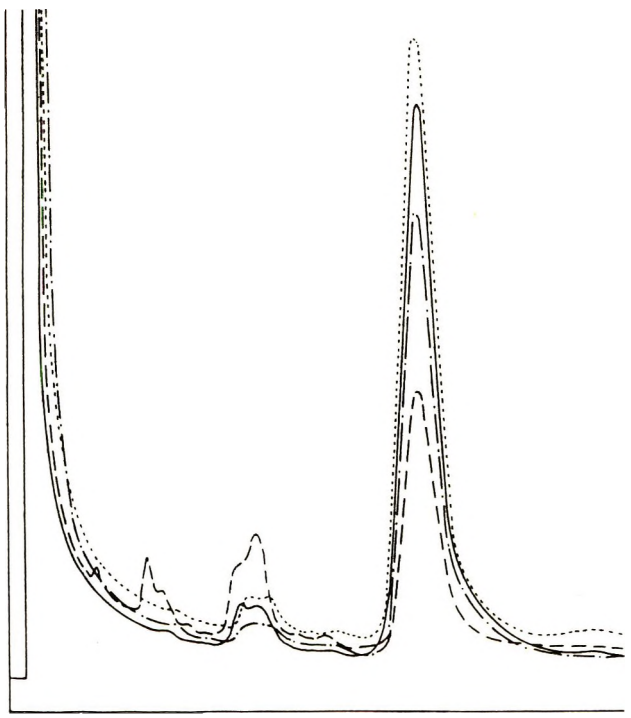


Figure 4. Comparative gas-liquid chromatograms of the oleyl alcohols. Temperature: 245°C. on 15% carbowax 20 M, 80/100 mesh chromosorb W. Temp. Inj.: 320°C. Col.: 2 m×⅛ in. (metal). - - - - - = /22/; ..... = /23/; ——— = /24/; —·—·— = /25/.

The investigation using gas chromatography has shown that substantial quantitative differences can exist between suppliers. By referring to the chromatograms, the following points can be remarked:

triglycerides (*Fig. 1*): the samples /4/ and /5/ give peaks practically superimposable whereas a slightly quantitative difference was noted with product /3/. Only two early peaks were obtained from the synthetic compound /6/.

isopropyl myristate (*Fig. 2*): the substances ref. /7/, /8/, /9/ present three peaks at C_{12}, C_{14}, C_{16} while n° /10/ has no peak at C_{12}.

isopropyl palmitate (*Fig. 3*): (/11/, /12/, /13/, /14/) comparable chromatograms are revealed (peaks at C_{14}, C_{16}, C_{18}) but the sample /11/ shows a greater number of peaks.

oleyl alcohol: the raw materials coded /23/, /24/ and /25/ are relatively pure compared to the n° /22/ (*Fig. 4*).

It was determined, at the conclusion of the experimentation, that most of the products stored at ambient temperature had a low peroxide index, with the exception of oleyl alcohols (n° /22/=20 mEq kg^{-1}, n° /23/=64 mEq kg^{-1}, n° /24/=41 mEq kg^{-1}, n° /25/= 56 mEq kg^{-1}); isopropyl palmitate n° /14/ (7 mEq kg^{-1}), decyl oleate n° /15/ (25 mEq/ kg) and arachidyl propionate n° /21/ (10 mEq kg).

Toxicological evaluation

The results given in *Tables II to VIII* call for several comments:

The column headed 'previous data' gives the toxicological information either of the supplier or found in the literature.

For the ocular irritation test, only the readings after 1 h, 24 h and 48 h have been recorded; in fact, with the exception of polyoxyethylene sorbitan stearate, the scores were zero for 3d, 4d and 7d. Also, due to the absence of any marked irritancy with undiluted products, no further tests were conducted.

For the primary skin irritation test, duplicate assays were monitored in many cases in order to ensure significant results.

For the 60-day cumulative irritation test, the data is a résumé of macroscopic and histological findings: they correspond to the most characteristic phenomena observed on all the animals.

Finally, the general conclusion is drawn on the tolerance for each product studied, taking into account the results of the entirety of the tests.

The interpretation was carried out using the following principles:

Ocular irritation index (*O.I.I.*): a compound does not provoke any significant injury to the eye mucous membrane when no clouding of the cornea occurs and when the ocular index is less than 10.

Primary irritation index (*P.I.I.*): the result is deemed satisfactory if the index is less than 0·5, but it is still acceptable if it is not greater than 1, taking into account experimental practice.

Cumulative irritation index (*C.I.I.*): concerning the skin response to repeated exposures, the interpretation is more complex because, for the series of products tested, after several applications, erythema was noted lasting until the final test. This erythema, scarcely visible for the diluted materials, was more serious when the products were tested undiluted. It was also frequently observed that vesicles appeared for spasmodic or prolonged periods, as well as papules, maculae or patches of erythema. However, if the

Table II. Squalane and hydrogenated polyisobutene

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores Macroscopic evaluation	Histological evaluation	Interpretation of tolerance†
Squalane /1/	Neat		1 h: 4·33 24 h: 0 00 48 h: 0 00	0·29	Relatively well tolerated M.M.I.I.* = 1·00 Presence of vesicles and papulae	Slight congestion of dermis probably of mechanical origin without pathological significance	+
	15%			assay n° 1: 0·00 assay n° 2: 0·00	Well tolerated M.M.I.I.* = 0·33 Presence of some vesicles	Normal	+
Hydrogenated /2/ polyisobutene	Neat	(supplier) O.I.I. = N.I. P.I.I. = 0·00 LD_{50} = 68·9 g/kg	1 h: 3·67 24 h: 2·67 48 h: 0·83	0·08	Relatively well tolerated M.M.I.I. = 0·67	Slight congestive lesions within the physiological limits	+
	15%			0·00	Well tolerated M.M.I.I. = 0·33 Presence of vesicles or macules	Normal	+

* M.M.I.I. = Mean maximum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.
N.I. = non-irritant.

Table III. Triglycerides

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores: Macroscopic evaluation	60-Day cumulative irritation scores: Histological evaluation	Interpretation of tolerance †
/3/	Neat	(supplier) LD_{50} > 25 ml/kg O.I.I.: NI	1 h: 4·00 24 h: 0·00 48 h: 0·00	0·21	Relatively well tolerated M.M.I.I.* = 0·83 Presence of vesicles in one rabbit	Superficial capillary dermal congestion within the physiological limits	+
	15%			0·08	Relatively well tolerated M.M.I.I. = 0·67 Presence of macules in one rabbit	No significant pathological reaction	+
/4/	Neat	(supplier) LD_{50} > 36 ml/kg O.I.I. = NI	1 h: 2·00 24 h: 0·00 48 h: 0·00	0·21	Relatively well tolerated M.M.I.I. = 0·67 Presence of vesicles in one rabbit	No significant pathological reaction	+
	15%	P.I.I. = NI		0 00	Relatively well tolerated M.M.I.I. = 0·67 Presence of macules in one rabbit	No significant pathological reaction	+
/5/	Neat		1 h: 4·00 24 h: 0 00	0·46	Poorly tolerated M.M.I.I. = 1·00 Presence of vesicles in the three animals	Two of the six biopsies showed pathological intra- and perifollicular inflammation, of the retention type	±
	15%			0·04	Relatively well tolerated M.M.I.I. = 0·67 Episodic vesicles in two rabbits	No significant pathological reaction	+
/6/	Neat	LD_{50} > 50 ml/kg	1 h: 0·71 24 h: 0·00 48 h: 0·00	0·29	Poorly tolerated M.M.I.I. = 1·00 Presence of vesicles in the three animals	Follicular reaction of inflammatory type, dermal congestion: pathological lesions	−
	15%			0·00	Relatively well tolerated M.M.I.I. = 0·82 Presence of vesicles in two rabbits	No significant pathological reaction	+

* M.M.I.I. = Mean manimum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table IV. Esters: isopropyl myristate

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores: Macroscopic evaluation	60-Day cumulative irritation scores: Histological evaluation	Interpretation of tolerance †
/7/	Neat	Publication (25): Suzuki	1 h: 4·33 24 h: 0·00 48 h: 0·00	assay n° 1: 1·00 assay n° 2: 1·00	Badly tolerated (2 assays, treatment stopped after 3 and 5 weeks) M.M.I.* = 1·67 thickening and serious drying of the skin with 'fissures'	Severe intolerance: spongiotic epidermis, hyperacanthosic with episodic papillomatose	−
	15%			assay n° 1: 0·00 assay n° 2: 0·20	Relatively well tolerated M.M.I.I. = 0·33 presence of vesicles in the three animals	No significant pathological reaction	+
/8/	Neat		1 h: 1·67 24 h: 0·00 48 h: 0·00	assay n° 1: 1·21 assay n° 2: 1·00	Badly tolerated (treatment stopped after 3 weeks) M.M.I.I. = 1·33 important epidermal exfoliation with 'fissures'	Severe intolerance: follicular suppuration	−
	15%			assay n° 1: 0·00 assay n° 2: 0·17	Well tolerated M.M.I.I. = 0·33 Presence of some vesicles	Normal	+
/9/	Neat		1 h: 4·17 24 h: 0·33 48 h: 0·00	assay n° 1: 0·25 assay n° 2: 0·08	Badly tolerated (treatment stopped after 3 weeks) M.M.I.I. = 1·33 epidermal exfoliation with 'fissures'	Pustulae in two animals probably from retention due to the fats	−
	15%			0·08	Relatively well tolerated M.M.I.I. = 0·50 presence of vesicles in two rabbits	No significant pathological reaction	+
/10/	Neat		1 h: 2·00 24 h: 0·00 24 h: 0·00	assay n° 1: 0·50 assay n° 2: 0·00	Badly tolerated (*8 weeks*) M.M.I.I. = 1·00 Thickening and drying of the skin with 'fissures'	Important thickening of epidermis with parakeratosis, congestive dermis with lymphocytic infiltrates	−
	15%			0·00	Relatively well tolerated M.M.I.I. = 0·33 presence of vesicles in two rabbits	No significant pathological reaction	+

*M.M.I.I. = Mean maximum irritation index.
†+ = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table V. Esters: isopropyl palmitate

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-day cumulative irritation scores Macroscopic evaluation	Histological evaluation	Interpretation of tolerance†
/11/	Neat		1 h: 3·33 24 h: 0·67 48 h: 0·00	assay 1: 0·96 assay 2: 0·50	Relatively well tolerated M.M.I.* = 1·00 Thickening of the skin, slight epidermal exfoliation	No significant pathological reaction	+
	15% (w: 1 to 5) 10% (w:6 to 8)			0·00	Relatively well tolerated M.M.I. = 0·83 Presence of vesicles in two of three rabbits	No significant pathological reaction	+
/12/	Neat		1 h: 6·50 24 h: 0·67 48 h: 0·00	assay 1: 1·25 assay 2: 0·96	Relatively well tolerated M.M.I. = 1·17 Thickening of the skin	Slight epidermal hyperacanthosis No pathological reaction	+
	15% (w:1 to 5) 10% (w:6 to 8)			0·04	Well tolerated M.M.I. = 0·50 Presence of vesicles in two rabbits	No significant pathological reaction	+
/13/	Neat		1 h: 5·83 24 h: 2·00 48 h: 0·00	assay 1: 0·25 assay 2: 0·42	Relatively well tolerated M.M.I. = 1·00 Thickening of the skin	Strateum corneum orthokeratosic Slight epidermal hyperacanthosis: reactions within the physiol. limits	+
	15% (w:1 to 5) 10% (w:6 to 8)			0·08	Relatively well tolerated M.M.I. = 0·67 Vesicles in only one animal	No significant pathological reaction	+
/14/	Neat		1 h: 5·33 24 h: 0·67 48 h: 0·00	assay 1: 0·33 assay 2: 0·33	Relatively well tolerated M.M.I. = 1·17 Thickening of the skin	Slight epidermal hyperacanthosis: reactions within the physiological limits	+
	15% (w:1 to 5) 10% (w:6 to 8)			0·04	Well tolerated M.M.I. = 0·67 Presence of vesicles in two rabbits	No significant pathological reaction	+

* M.M.I. = Mean maximum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table VI. Esters—miscellaneous

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores Macroscopic evaluation	Histological evaluation	Interpretation of tolerance†
Decyl /15/ oleate	Neat		1 h: 2·67 24 h: 3·00 48 h: 0·33	0·13	Poorly tolerated M.M.I.I.* = 1·00 Skin thickening in the three rabbits, vesicles in one animal	Tathological reaction: congestive dermis	−
	15%			0	Relatively well tolerated M.M.I.I. = 0·33 Some papulae or vesicles	No significant pathological reaction	+
Isodecyl /16/ oleate	Neat		1 h: 4·00 24 h: 0·00 48 h: 0·00	assay 1: 0·13 assay 2: 0·00	Poorly tolerated M.M.I.I. = 1·00 Vesicles in one animal	Congestive dermis with peri-capillary inflammatory infiltrates: pathological reaction	−
	15%			assay 1: 0·00 assay 2: 0·00	Relatively well tolerated M.M.I.I. = 0·50 Episodical macules, papulae and vesicles	No significant pathological reaction	+
Octyl /17/ palmitate	Neat	(supplier) O.I.I. = N.I. P.I.I. = 1·6 DL_{50} 8ml/ kg	1 h: 4·17 24 h: 0·00 48 h: 0·00	0·08	Poorly tolerated in two animals M.M.I.I. = 0·83 Vesicles in two rabbits	Adverse reactions (congestive dermis) for three of the six biopsies	±
	10%			0·00	Well tolerated M.M.I.I. = 0·33 Vesicles in two rabbits	Normal	+
Octyl /18/ stearate	Neat		1 h: 4·67 24 h: 0·00 40 h: 0·00	0·00	Poorly tolerated M.M.I.I. = 0·67 Vesicles in the three rabbits slight epidermal exfoliation	Epidermal hyperacanthosis, congestive dermis for all the animals	−
	10%			0·00	Relatively well tolerated M.M.I.I. = 0·33 Vesicles in two rabbits	No significant pathological reaction	+

* M.M.I.I. = Mean maximum irritation index
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table VII. Esters—miscellaneous

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores Macroscopic evaluation	Histological evaluation	Interpretation of tolerance†
Cetearyl /19/ octoate	Neat	(supplier) O.I.I. (24 h): 0·78 P.I.I.: 0·0 C.I.I. (90 days) =1·43	1 h: 8·17 24 h: 0·33 48 h: 0·00	0	Poorly tolerated M.M.I.I.* =0·83 Exfoliation, slight thickening, vesicles, maculae or papules	Orthokeratosic flattened stratum corneum and epidermal hyperacanthosis: pathol. reactions	−
	10%			0	Relatively well tolerated M.M.I.I. =0·33 Vesicles in the three animals	No significant pathological reaction	+
Stearyl /20/ heptoate	Neat		1 h: 6·00 24 h: 0·00 48 h: 0·00	0·13	Relatively well tolerated M.M.I.I. =0·67 Presence of vesicles and/or erythematous patches	Moderate epidermic hyperacanthosis, slight congestive dermis: within physiol. limits	+
	10%			0	Well tolerated M.M.I.I. =0·17 Vesicles in one rabbit	Normal	+
Arachidyl /21/ propionate	Neat	(supplier) LD_{50} > 20 g/kg O.I.I.: N.I. P.I.I.: N.I.	1 h: 3·83 24 h: 1·50 48 h: 0·00	0·13	Relatively well tolerated M.M.I.I. =1·00 Slight epidermal exfoliation	Very slight congestion of the dermis: no pathol. reaction	+
	10%			0	Well tolerated M.M.I.I. =0·50 Vesicles in two rabbits	No significant pathological reaction	+

* M.M.I.I. = Mean maximum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table VIII. Oleyl alcohol

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores: Macroscopic evaluation	60-Day cumulative irritation scores: Histological evaluation	Interpretation of tolerance†
Oleyl alcohol /22/	Neat		1 h: 7·17 24 h: 0·33 48 h: 0·00	assay 1: 1·71 assay 2: 1·58	Badly tolerated M.M.I.I.* = 1·33 Important thickening and drying of the skins, eschars	Flattened stratum corneum with hyperacanthosis in the three rabbits	−
	10%			assay 1: 0·17 assay 2: 0·33	Relatively well tolerated M.M.I.I. = 0·50	Slight non-inflammatory hyperplasis with moderate hyperacanthosis and vascular congestion of the superficial dermis	+
Oleyl alcohol /23/	Neat		1 h: 5·83 24 h: 3·17 48 h: 0·66	assay 1: 1·67 assay 2: 1·75	Badly tolerated M.M.I.I. = 1·50 Important thickening and drying of the skin, eschars	Important hyperacanthosis with flattened strat. corneum	−
	10%			assay 1: 0·04 assay 2: 0·25	Relatively well tolerated M.M.I.I. = 0·67 Slight epidermal exfoliation	Slight congestion without inflammatory infiltrates or epidermic alteration	+
Oleyl alcohol /24/	Neat		1 h: 5·67 24 h: 3·67 48 h: 2·00	1·50	Badly tolerated M.M.I.I. = 1·50 Thickening and drying, 'fissures', eschars	Flattened stratum corneum hyperacanthosis of the epidermis	−
	10%			0·29	Relatively well tolerated M.M.I.I. = 0·75 Slight epidermic exfoliation	Slight congestive dermis, within the physiol. limits.	+
Oleyl alcohol /25/	Neat		1 h: 5·00 24 h: 0·67 48 h: 0·00	1·33	Badly tolerated M.M.I.I. = 1·50 Thickening drying, 'fissures', eschars	Orthokeratosic stratum corneum, hyperacanthosis of the epidermis with episodic papillomatosis	−
	10%			0·42	Relatively well tolerated M.M.I.I. = 0·83 Slight epidermic exfoliation	Slight erythema with stratum corneum slightly flattened; within the physiol. limits	+

* M.M.I.I. = Mean maximum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

Table IX. Polyoxyethylene sorbitan stearate (Polysorbate)

Compound	Concentration	Previous data	Irritation of the ocular mucous membrane O.I.I.	Primary irritation of the skin P.I.I.	60-Day cumulative irritation scores Macroscopic evaluation	Histological evaluation	Interpretation of tolerance†
Polyoxyethylene sorbitan stearate /26/	Neat	(supplier) P.I.I.: 0·46–0·54	1h: 8·17 1d: 3·17 2d: 1·67 3d: 1·33 4d: 0·33 7d: 0·00	0·29	Relatively well tolerated M.M.I.I.* = 0·67 (No other lesion observed)	Slight congestion of the dermis within the physiol. limits	+
	15%			0·00	Very well tolerated M.M.I.I. = 0·17 (no other cutaneous lesion)	Normal	+

* M.M.I.I. = Mean maximum irritation index.
† + = Good tolerance; − = Bad tolerance; ± = Doubtful tolerance.

presence of vesicles is an allergic reaction in the human, it would appear that this is not applicable to the rabbit, because in this event 60–70% of cosmetics would appear to contain allergy-inducers.

It appears that this is due to an occlusive film formed by the remaining material, in spite of all the precautions taken to remove the excess of product. The pores in the skin of the rabbit are more dilated than those of human skin and materials tend to accumulate in these cavities.

Furthermore, these products when applied under the same conditions to hairless or normal rats, rarely provoke the appearance of vesicles.

To ensure a proper evaluation of skin irritancy it is necessary that macroscopic observations show comparable results on all the animals involved because primary irritation reactions give a collective response contrary to sensitisation. It is important, also, that reactions are observed over the total epidermis tested and not just at localized points.

Finally, the histological examinations carried out on two biopsies for each rabbit should confirm the macroscopic observations.

Because the rabbit skin is more responsive than human skin, this procedure is necessary to eliminate individual or local reactions and to take into consideration only the pathological lesions.

DISCUSSION

None of the materials tested provoked pathological lesions of the ocular mucous membrane (such as corneal opacification, ulceration . . .) The observed anomalies are benign, probably of mechanical origin and correspond mainly to congestion of the iris, conjunctival enanthema, and a slight discharge and/or chemosis.

The highest ocular index was 8·17 (slightly irritant) for polyoxyethylene sorbitan stearate /26/ ceto-stearyl octoate /19/.

All the indices of primary skin irritation were less than 2; the maximum index of 1·75 (slightly irritant) was found with the oleyl alcohol n° /23/ tested undiluted.

The summarized data shows that the four oleyl alcohols gave adverse reactions when applied pure without rinsing (mean score=1·55/s=0·17); two isopropyl myristates /7/ and /8/, and one isopropyl palmitate /12/ have a score in the region of 1 (slightly irritant).

All these results were confirmed by two assays. The other products show a good tolerance particularly when studied as 10–15% solutions without rinsing, as did the diluted oleyl alcohols (average score: 0·28).

It was noted that for eleven of the twenty-six raw materials no adverse reactions were obtained after daily application to rabbit epidermis of the undiluted chemical. These are:

Squalane /1/ and the substitute polyisobutylene /2/
Two samples of caprylic/capric triglycerides /3/ and /4/
The four samples of isopropyl palmitate /11/ to /14/ (a skin thickening was noted macroscopically but the histological examination did not reveal any pathological effect.)
Stearyl heptoate /20/
Arachidyl propionate /21/
Polyoxyethylene sorbitan stearate /26/

On the contrary, eight products were harmful:

The four samples of oleyl alcohol /22/ to /25/
The four samples of isopropyl myristate /7/ to /10/

Of the remaining seven products, two give uncertain results (capric/caprylic triglyceride /5/ and octyl palmitate /17/) and for the others the irritation level was moderate (synthetic triglyceride /6/, decyl oleate /15/, isodecyl oleate /16/, octyl stearate /18/ and cetostearyl octoate /19/).

On the whole, the products were well tolerated when diluted to 10–15%, a figure which corresponds to the concentration found generally. In particular, the histological examination was negative. It should be noted, however, that the four samples of I.P.M. provoke the appearance of numerous vesicles. This oil, used for many years and even at high levels in many cosmetic preparations, gave a high irritation potential. It can induce an acanthosis and an oedematous degeneration of collagen fibres when applied under an occlusive patch to the rabbit for 24 h (25). Nevertheless, experiments conducted on miniature pigs do not show adverse results.

The macroscopic observations are characteristic of primary irritant reactions with a degree of erythema and sometimes thickening or drying of the skin. The mean results of the weekly average show a maximum for I.P.M. n° /7/ (1·67) and oleyl alcohols n° /23/, /24/, /25/ (1·50). These counts are relatively low (maximum rate=8) but this is due to the fact that they are calculated taking into account the measurements of erythema and oedema. However, oedema, rarely evident in histology, was never observed macroscopically.

On the other hand, secondary reactions such as vesicles, papules, maculae and/or erythematous zones were frequently noted. Furthermore, when intense aggravation occurs, 'fissures' can form and give rise to eschars which can be infectious.

The microscopic findings confirm generally the macroscopic scores. Unfortunately, in cases of too severe intolerance, histology cannot serve to distinguish the irritant reactions from the allergies.

It should be noted that the challenge assay, carried out after a rest period, revealed no real allergen (this was predictible considering the nature of the products studied).

The analytical investigations cannot explain, for the time being, the origin of the phenomena observed. There is, however, a possible relation between tolerance and purity for I.P.M.

It is hoped these experimental results can be of use to the cosmetic investigator, in spite of the fact that extrapolation to the human response is delicate.

ACKNOWLEDGMENTS

All the histological examinations were performed and interpreted by Dr J. Guilaine, Dermatologist. The authors also gratefully acknowledge the assistance of: J. Y. Guyot, J. P. Petit, G. Blanc, J. Charroy, B. Audenaerde, J. Evans, F. Gaubert, D. Thiry and L. Ferrero.

REFERENCES

1 Baer, R. L., Ramsey, D. L. and Biondi, E. The most contact allergens 1968–1970. *Arch. Dermatol.* **108** 74–78 (1973).

2 Bandmann, H. J. and Breit, R. Chemically induced allergic contact dermatitis. *Intermist* (*Berlin*) **15** (1) 47–51 (1974).
3 Breit, R., Bandmann, H. J. Contact dermatitis XXII. Dermatitis from Lanolin. *Brit. J. Dermatol.* **88** (4) 414–416 (1973).
4 Brulos, M. F., Guillot, J. P., Martini, M. C. and Cotte, J. Mise au point d'une méthode d'évaluation du pouvoir sensibilisant, par applications topiques, chez le cobaye. Comptes rendues des séances de la Société de Biologie et de ses filiales. Communication présentée à la 11e semaine internationale de Dermocosmétologie (18 janvier 1977 à Lyon). A paraître dans *J. Cosmet. Chem.*
5 Cronin, E. Contact dermatitis from cosmetics. *J. Soc. Cosmet. Chem.* **18** 681–691 (1967).
6 Epstein, E. The detection of lanolin allergy. *Arch. Dermatol.* **106** (5) 678–681 (1972).
7 Fallon, P. Dermatitis litigation. *Proc. R. Soc. Med.* **65** (12) 1071–1078 (1972).
8 Feuerman, E. Editorial: allergic skin reaction to lanolin. *Harefuah,* **86** (7) 380–381 (1974).
9 Fischer, A. The use of patch-test series. In: *Contact Dermatitis*, 2nd Ed. Lea & Febiger, Philadelphia (1973).
10 Foussereau, J. La multiplication actuelle des substances nocives en dermato-allergie. *Strasbourg Med.* **15** 704–715 (1964).
11 Foussereau, J. and Basset, A. La sensibilisation aux excipients et conservateurs de pommades. *Concours Med.* **11** 1063–1068 (1967).
12 Gaul, L. E. Dermatitis from stearyl and cetyl alcohols. *Arch. Derm.* **99** 598 (1969).
13 Hardy, J. Allergy, hypersensitivity and cosmetics. *J. Soc. Cosm. Chem.* **24** 423–468 (1973).
14 Huriez, C., Martin, P. and Vanowerschelde, M. L'allergie au propylène glycol. *Bull. Soc. Franc. Derm. Syphl.* **73** 263–267 (1966).
15 Journal Officiel de la République Française du 21/4/71, édition Lois et Décrets, et du 5/6/73, ed. Documents administratifs—Méthodes officielles d'Analyse des cosmétiques et produits de beauté.
16 Kinmont, P. D. C. Skin reactions to cosmetic preparations. *J. Soc. Cosmet. Chem.* **15** 3–32 (1964).
17 Martini, M. Cl. Les produits cosmétiques. Toxicologie clinique et analytique Législation. III: les produits de beauté et les parfums. *Lyon-Pharmaceutique* **25** (3) 279–303 (1974).
18 Marzulli, F. N. and Ruggles, D. I. Rabbit eye irritation rest: collaborative study. *J. of the AOAC.* **56** (4) 905–914 (1973).
19 Marzulli, F. N. and Maibach, H. A. The rabbit as a model for evaluating skin irritants: a comparison of results obtained on animals and man using repeated skin exposures. *Food Cosm. Toxicol.* **13** 553–540 (1975).
20 Masters, E. J. Allergies to cosmetic Products—Symposium cosmetic allergy, N.Y. *N.Y. State J. Med.* 1934–1940 (1960)
21 Meneghini, C. L., Rantuccio, F. and Lomuto, M. Additives, vehicles and active drugs of topical medicament as causes of delayed type allergic dermatitis. *Dermatologica* **143** 137–147 (1971).
22 Phillips, L., Steinberg, M. and Maibach, H. I. A comparison of rabbit and human skin response to certain irritants. *Toxicol. & Appl. Pharm.* **21** 369–382 (1972).
23 Schorr, W. F. Contact dermatitis. Office diagnosis and management. *Minn. Med.* **57** (10) 831–837 (1974).
24 Shore, R. N. and Shelley, W. B. Contact dermatitis from stearyl alcohol and propylene glycol in fluocinonide cream. *Arch. Dermatol.* **109** (3) 397–399 (1974)
25 Suzuki, M. *et al.* Autoradiographic study on percutaneous absorption of several oils in cosmetic field. *I.F.S.C.C.* (1976).
26 Thomas, M. J. and Majors, P. A. Animal, human and microbiological safety testing of cosmetic products. *J. Soc. Cosmet. Chem.* **24** 135–146 (1973).
27 Van Abbe, N. J. Eye irritation: studies relating to responses in man and laboratory animals. *J. Soc. Cosmet. Chem.* **24** 685–692 (1973).
28 Van Abbe, N. J., Nicholas, P. and Boon, E. Exaggerated exposure in topical irritancy and sensitization testing, *J. Soc. Cosmet. Chem.* **26** 173–187 (1975).
29 Van Hecke, E. A case of dichromate dermatitis. *Arch. Belg. Dermatol. Syphi.* **28** (4) 405–408 (1972).
30 Wollmann, C., Meyer, F. U. Schwerke, W. Animal experiments studies on the effect of various ointment bases on the epidermis. II: Studies on the effect of vaselinum flavum and various batches of cera liquida on the epidermis width and mitosis. *Dtsch Gesundheitsw* **27** (33) 1571–1575 (1972).
31 Zina, G. and Bonu, G. Importance des divers composants des excipients dans l'allergie de contact aux topiques utilisés en médecine. *La Revue de la Médecine* **28** 1639–1644 (1970).

J. Soc. Cosmet. Chem. **28** 395–401 (1977)

The mechanism of skin pigment production

P. A. RILEY *Department of Biochemical Pathology, University College Hospital Medical School, University Street, London WC1 E6JJ*

Presented at the Joint Symposium with the Pharmaceutical Society of Great Britain "Cosmetic and Pharmacological Aspects of Colour" 9–11 November 1976, at Stratford upon Avon.

Synopsis

The general metabolic pathways leading to the production of **pheomelanins** and **eumelanins** are outlined. This paper surveys the evidence that two types of oxidation by **tyrosinase** are involved, namely oxygen addition to **monophenols** (**cresolase** activity) and dehydrogenation of **diphenols** (**catecholase** activity). Highly reactive **quinones** are formed as intermediate metabolites and it is suggested that they are of importance as possible sources of perturbation of cell metabolism.

DEFINITION OF MELANIN

The most widespread surface pigment in the animal kingdom is melanin. The term 'melanin' was first applied by Berzelius in 1830 to mean any dark pigment, without any specific chemical implications other than those of relative insolubility. Subsequent attempts to derive a more specific chemical definition have foundered on the difficulty that the detailed structures of most melanoid pigments are unknown. Indeed, the structures which have been suggested for melanins have largely been pigments of the imagination. An important fact about melanins is that they absorb light over a large portion of the spectrum. A definition based on their spectral properties is probably the most useful.

The biological importance of melanins is related to the fact that they absorb light throughout the visible spectrum and that means that the light absorbed includes radiation which has very low quantal energy and therefore only small electron energy transitions are involved. The smallest energy transitions are from non-bonding to anti-bonding *pi*-orbitals ($n \rightarrow \pi^*$) and these occur in amide and carbonyl bonds. Melanins also strongly absorb in the ultraviolet spectrum and this involves transitions of electrons from bonding to anti-bonding *pi*-orbitals ($\pi \rightarrow \pi^*$). *Pi*-orbitals occur in unsaturated carbon-bonds, and transitions from bonding to anti-bonding orbitals are facilitated by conjugation, especially when the *pi*-electrons are delocalised, as in aromatic rings. The effect of this is that, as the degree of conjugation increases, lower and lower quantal energies are required for absorption. This effect is called *bathochromicity*. Thus, we may define melanins as bathochromic aromatic substances which contain oxygen and nitrogen.

Melanins are quinonoid polymers of somewhat uncertain structure. They are generally classified according to their predominant component which in most cases is an oxidation

product of the amino acid tyrosine. Two major subdivisions are recognised: *pheomelanins* and *eumelanins*. The pheomelanins are distinguished from eumelanins by their content of sulphur. They are derived from a combination of tyrosine oxidation products with cysteine which give rise to yellowish trichochromes of small molecular weight (<1000 daltons), and reddish brown macromolecular pigments. Eumelanins, by contrast, are black or brown insoluble pigments derived from the polymerisation of tyrosine oxidation products.

THE METABOLIC PATHWAY OF MELANOGENESIS

The essential steps in melanogenesis consist of the enzymatic oxidation of tyrosine and its derivatives, linked to a series of spontaneous reductions. The initial oxidation consists of the addition of oxygen in the *ortho* position of the aromatic ring (*Fig. 1*). The oxidation product which is formed is 3,4-dihydroxyphenylalanine (dopa). Dopa, in common with most diphenols, readily undergoes oxidation to give rise to the corresponding quinone: the reaction consists of a dehydrogenation which is also catalysed by the enzyme tyrosinase. This dehydrogenation is an oxygen-requiring reaction and the oxidation of the diphenolic substrate is linked to the reduction of molecular oxygen to water. As far as the dehydrogenation reaction is concerned tyrosinase shows relatively low substrate specificity with respect to the side chain. It has been shown that 5,6 dihydroxyindole is oxidised by tyrosinase and some non-physiological substrates are also oxidised, some of which (such as the hydroxylated derivatives of anisole) may have therapeutic implications. Where dopa is the substrate the product is dopa quinone (*Fig. 2*).

Figure 1. Oxidation of tyrosine to dopa.

Figure 2. Dehydrogenation of dopa to form dopa quinone.

Like most quinones, dopa quinone is a highly reactive molecule, a feature which is made use of in the defensive sprays of some arthropods (1). Quinones readily condense with amino groups and will form cross-links with proteins; and the reactivity of quinones may also generate undesirable deleterious effects if unrestricted access is permitted to potential substrates in the cell such as membrane lipids (see below). The spontaneous reduction steps involved in the generation of tyrosine oxidation products consist of four major reactions: reductive cyclisation, redox exchanges, reductive addition and reductive polymerisation.

Indolene formation occurs by the addition of the side chain amino group to the sixth ring carbon with simultaneous reduction of dopa quinone. This gives rise to cyclo-dopa

(*Fig. 3*). This compound is probably identical to the leucodopachrome of Raper (2). Under appropriate conditions there is a spontaneous rearrangement of cyclo-dopa with the loss of carbon dioxide and two hydrogen atoms to give 5,6 dihydroxyindole (*Fig. 4*). There is some evidence to suggest that this dehydrogenation is achieved by a redox exchange with a quinone intermediate. In the absence of this step alternative additive reactions may give rise to oligomers containing cyclo-dopa (3).

Figure 3. Cyclization of dopa quinone to give the indolene product cyclo-dopa (leucodopachrome).

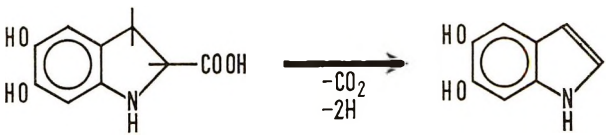


Figure 4. Rearrangement of cyclo-dopa to give 5,6-dihydroxyindole.

In addition to catalysing the conversion of cyclo-dopa the quinone intermediates, such as dopa quinone and indole 5,6 quinone, may take part in the oxidation of compounds which cannot be directly oxidized by tyrosinase by virtue of their structure or their location in relation to the enzyme. Some of these redox reactions may be extremely deleterious to the pigment-producing cells (for discussion see ref. 1). In man at least one mechanism is known to exist which traps the reactive quinones and prevents them from initiating cellular damage. The trapping reaction consists of the formation of a reduced C-5 adduct with glutathione. The glutamic acid and glycine residues are cleaved by peptidases giving rise to 5-S-cysteinyl dopa, which can be detected in melanocytes and in the serum, and is excreted in the urine (4).

The melanin group at Naples (5) have shown that cysteinyl dopa can also be formed by a direct interaction between dopa quinone and cysteine (*Fig. 5*).

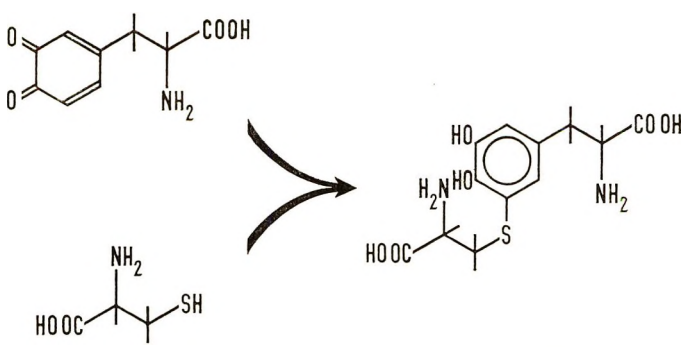


Figure 5. Condensation of dopa quinone with cysteine to form 5-S-cysteinyl dopa.

Two isomers are formed *in vitro*: 2-cysteinyl dopa and 5-cysteinyl dopa, the latter in proportionately much larger amounts. If the product is oxidized to cysteinyl quinone spontaneous cyclization takes place with reduction to dihydrobenzothiazine (*Fig. 6*). This reaction is linked to the oxidation of a diphenol such as cysteinyl-dopa so that the generation of dihydrobenzothiazine, once initiated, can proceed non-enzymatically. This may explain the apparently paradoxical findings of Prota's group (6) that 3-^{14}C-cysteine incorporation by pigment cells is related to tyrosinase activity whereas Geschwind, Huseby and Nishioka (7) showed that MSH administration to A^Y mice switched melanocytes from pheomelanin to eumelanin production.

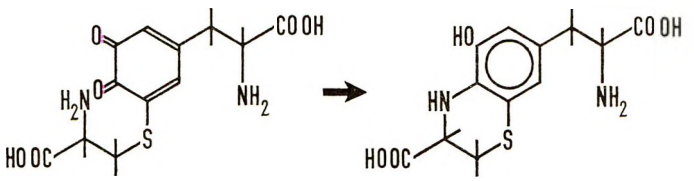


Figure 6. Reduction of 5-S-cysteinylquinone to form dihydrobenzothiazine.

It has been shown that the pheomelanins are composed of more or less complex polymers of dihydrobenzothiazine. A number of trichochromes which are composed of dimers of dihydrobenzothiazine have been identified and degradation studies of gallo-pheomelanin obtained from New Hampshire hen feathers have shown it to be an irregular and complex polymer perhaps containing benzothiazole and tetrahydroisoquinoline ring systems.

A similar lack of simplicity is evident in the composition of eumelanins. Polymerization of the oxidation products of tyrosine takes place by a series of reductive additions. A random assortment of oxidized intermediates is incorporated into the polymer which therefore lacks any specific structure. The most regular structure is one produced by polymerization between C4 and C7 atoms of adjacent indole quinone rings. X-ray diffraction studies indicate that stacking of the rings occurs forming a lattice arrangement and this constraint may be responsible for introducing some regularity into the polymer by restricting access to sterically affine structures. Two major differences between eumelanins and pheomelanins emerge from a knowledge of their biosynthesis. Pheomelanins, because they are polymers of less conjugated precursor molecules, show a much more restricted spectral absorbance than eumelanins and thus give the structures which they pigment a reddish or yellowish appearance in contrast to the dark brown or black of the highly conjugated eumelanins. Secondly, because the cyclization of cysteinyl-quinone replaces one of the hydroxyl groups on the phenol ring, the pheomelanin polymer cannot contain reactive quinones and this prevents the formation of cross-linkages with protein and leads to the characteristic lack of organization within pheomelanin pigment granules.

In the case of eumelanins the protein tanning effect of quinone constituents of the pigment is very marked and seems to be of importance in the hardening of insect cuticles and sclerotization reactions in other invertebrates (1). In mammalian melanosomes the reaction of eumelanin quinones with protein probably accounts both of the melanin deposition on the matrix and the inhibition of tyrosinase in the fully melanized granule. The highly conjugated structure of eumelanins permits electron movements and electron exchanges with neighbouring molecules take place readily, i.e. melanins are chemically highly reactive. Commoner, Townsend and Pake (8) first demonstrated the paramagnetic

properties of melanins and subsequently Mason's group (9) showed that the free radical property of melanin is due to semiquinones, stabilized by resonance in the highly conjugated polymer and steric restrictions on internal radical annihilation reactions. Molecular orbital calculations by Pullman and Pullman (10), based on an assumed structure for dopa-melanin, showed that several redox states are possible and predicted electron acceptor properties which have been confirmed by several studies (11, 12, 13, 14).

This reactivity of melanins may be the reason which necessitates its compartmentation in membrane-bounded melanosomes in the melanocytes (15). When transferred to the cytoplasm of keratocytes, melanin granules probably act as initiators of cytoplasmic damage in cells exposed to radiation which is absorbed by the pigment and generates free radicals (9, 16). The evidence for this proposal is discussed elsewhere (1, 17, 18) but it is probable that the selective advantage to hairless mammals is the destruction of cells which have received a radiation dosage sufficient to cause deleterious mutations. Clearly, from what has been said about their structure, the pheomelanins are much less effective in this respect and this is borne out by the statistics on the susceptibility of various ethnic groups to skin cancer.

TYROSINASE

The enzyme responsible for the oxidations involved in melanogenesis is tyrosinase. At least two forms (α and β) of the enzyme are recognized and minor differences in activity exist but the general properties are broadly identical. They are widespread in nature, occurring in both eukaryotic and prokaryotic organisms. In vertebrates the enzyme is synthesized only in specialized cells (melanocytes) and is active only in specialized cytoplasmic organelles (melanosomes). In contrast to many other enzymes whose structures have been determined and for which the catalytic mechanisms are well understood, tyrosinase is still very poorly comprehended and no clear reaction mechanism has emerged. It is known that tyrosinases contain copper and that they bind oxygen. There are two main classes of oxidation catalysed by tyrosinases: the dehydrogenation of diphenols (catecholase activity) and the ortho-hydroxylation of monophenols (cresolase activity).

CATECHOLASE ACTIVITY

The enzyme isolated from *Neurospora crassa* has been studied by a number of groups. The enzyme has a molecular weight of about 33 000 daltons and contains one atom of copper per mole of protein (19). The kinetic studies of Gutteridge and Robb (20) indicate a single binding site for diphenolic substrates. Electron spin resonance studies show that only 4% of the copper is in the cupric form and spectral absorbance data indicate that 35% is complexed with monomolecular oxygen leaving about 60% of the enzyme free to combine with the substrate. Binding to substrate and oxygen is thought to take place in a random sequence (*Fig. 7*). The *Neurospora* enzyme appears to be incapable of oxidizing monophenols.

CRESOLASE ACTIVITY

Mushroom (*Agoricus bisporus*) tyrosinase is a tetramer of 130 000 daltons molecular weight and contains four atoms of copper. On the basis of the reaction with hydrogen peroxide, Jolley *et al.* (21) have proposed a dimeric active site containing two copper

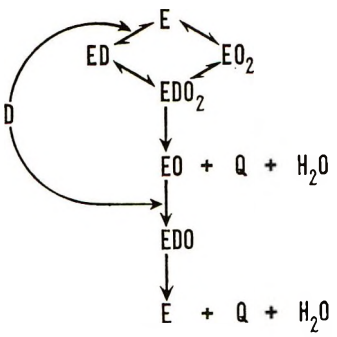


Figure 7. Schematic outline of catecholase activity of *Neurospora* tyrosinase (after Gutteridge and Robb (20). E = enzyme; D = diphenol; Q = quinone.

atoms. Mason (22) furnished evidence that monophenol oxidation involves the transfer of two electrons to generate the cupric form of the enzyme. If this is confirmed it suggests that cresolase activity is consequent upon aggregation of monomeric catecholases of the type found in *Neurospora* and *Streptomyces glaucescens* (23). This leads to the interesting speculation that monophenol oxidation by tyrosinase could be controlled by factors limiting the extent of subunit interaction.

MAMMALIAN TYROSINASE

Studies on mammalian tyrosinase employing SDS poly-acrylamide electrophoresis by Burnet's group (24) have shown that the smallest sub-unit capable of oxidizing dopa has a molecular weight of roughly 65 000 daltons which would correspond in size to the dimer of the *Neurospora* enzyme. In some instances another component with a molecular weight of about 120 000 daltons can also be detected which is about the size of the tetramer of the mushroom enzyme. However, a principal component in the material obtained from mice has a molecular weight of about 80 000 daltons and it is probable that this is a form of tyrosinase which is modified by interaction with another protein component. The structure of tyrosinase is under the control of the C (or colour) locus. Modifying loci, such as pink-eyed dilution and dilute, seem to have the effect of converting more of the enzyme into the modified form. It may be that the coloration of animals carrying these genes is, therefore, modified by an interaction of tyrosinase with peptides which may cause a reduction in cresolase activity, possibly as a result of a separation of the copper atoms in the dimer. Such a modification would not affect diphenol oxidation but, by inhibiting the first step in tyrosinase oxidation, reduce overt pigmentation.

REFERENCES

1 Riley, P. A. The mechanisms of melanogenesis. *Symp. Zool. Soc. Lond.* **39** 77 (1977).
2 Raper, H. S. The aerobic oxidases. *Physiol. Revs.* **8** 245 (1928).
3 Gruhn, W. B., Pomeroy, J. S. and Maurer, L. M. An oligomeric hydroxyphenylalanine in malignant melanoma: a new type of melanogen. *Biochem Biophys. res. Comm.* **61** 704 (1974).
4 Rorsman, H. The melanocyte illuminated. *Trans. St John's Hosp. Derm. Soc.* **60** 135 (1974).
5 Prota, G. and Thomson, R. H. Melanin pigmentation in mammals. *Endeavour* **35** 32 (1976).
6 Misuraca, G., Nicolaus, R. A., Prota, G. and Gliara, G. A cytochemical study of pheomelanin formation in feather papillae of New Hampshire chick embryos. *Experientia* **25** 920 (1969).
7 Geschwind, I., Huseby, R. A. and Nishioka, R. *Recent Progress in Hormone Research* Vol. **28**, pp. 91–130, Academic Press, N.Y. (1972).
8 Commoner, B., Townsend, J. and Pake, G. E. Free radicals in biological materials. *Nature* **174** 689 (1954).

9 Mason, H. S., Ingram, H. E. and Allen, B. Free radical property of melanins. *Arch. Biochem. Biophys.* **86** 225 (1960).
10 Pullman, A. and Pullman, B. The band structure of melanins. *Biochem. biophys. Acta* **54** 384 (1961).
11 Van Woert, M. H. Oxidation of reduced nicotinamide adenine dinucleotide by melanin. *Life Sci.* **6** 2605 (1967).
12 Van Woert, M. H. Reduced nicotinamide-adenine dinucleotide oxidation by melanin: inhibition by phenothiazines. *Proc. Soc. Exp. Biol. Med.* **129** 165 (1968)
13 Gan, E. V., Haberman, H. F. and Menon, I. A. Electron transfer properties of melanin. *Arch. biochem. biophys.* **173** 666 (1976).
14 Sarna, T., Hyde, J. S. and Swartz, H. M. Ion-exchange in melanin: An electron spin resonance study with lanthanide probes. *Science.* **192** 1132 (1976).
15 Slater, T. F. and Riley, P. A. Photosensitisation and lysosomal damage. *Nature* **209** 151 (1966).
16 Pathak, M. A. and Stratton, K. Free radicals in human skin before and after exposure to light. *Arch. Biochem. biophys.* **123** 468 (1968).
17 Riley, P. A. Dendritic Cells of the Epidermis. In: *Physiology and Pathophysiology of the Skin* (ed. A. Jarrett) pp. 1101–1235. Academic Press, London (1974).
18 Proctor, P., McGinness, J. and Corry, P. A hypothesis on the preferential destruction of melanised tissues. *J. theoret. biol.* **48** 19 (1974).
19 Fling, M., Horowitz, N. H. and Heinemann, S. F. The isolation and properties of crystalline tyrosinase from *Neurospora. J. Biol. Chem.* **238** 2045 (1963).
20 Gutteridge, S. and Robb, D. The catecholase activity of *Neurospora* tyrosinase. *Eur. J. Biochem.* **54** 107 (1975).
21 Jolley, R. L., Evans, L. M., Makino, N. and Mason, H. S. Oxytyrosinase. *J. Biol. Chem.* **249** 335 (1974).
22 Mason, H. S. The structure and functions of the phenolase complex. *Nature* **177** 79 (1956).
23 Lerch, K. and Ettlinger, L. Purification and properties of a tyrosinase from *Streptomyces glaucescens. Pathol. microbiol.* (Basel) **38** 23 (1972).
24 Holstein, T. J., Quevedo, W. C. and Burnett, J. B. Multiple forms of tyrosinase in rodents and lagomorphs with special reference to their genetic control in mice. *J. exp. Zool.* **177** 173 (1971).

J. Soc. Cosmet. Chem. **28** 403–406 (1977)

Enhancement of pigmentation: psoralens

RODNEY P. R. DAWBER *Consultant Dermatologist, Radcliffe Infirmary and Slade Hospital, Oxford*

Presented at the Joint Symposium with the Pharmaceutical Society of Great Britain "Cosmetic and Pharmacological Aspects of Colour" 9–11 November 1976, at Stratford upon Avon.

Synopsis

Biological extracts of various common plants have been used in depigmenting conditions for many centuries to enhance **pigmentation.** Specific photodynamic chemicals have been extracted from such sources—**psoralens.** Synthesis of these substances in 1947 led to detailed laboratory and clinical investigation of their mode of action and effectiveness in increasing normal pigmentation and repigmenting vitiliginous skin. The theoretical fear that long-term psoralen and **ultraviolet radiation** treatment might induce skin tumours has not so far been realised in practice.

INTRODUCTION

Psoralens belong to a group of heterocyclic compounds known as furocoumarins and are found in many edible plants, e.g. celery, caraway and figs. They have formed the basis of many herbal remedies used in recent centuries, mostly extracted from the two plant families Umbelliferae and rutaceae, though furocoumarins are somewhat ubiquitous throughout the plant kingdom. These substances appear to have a variety of physiochemical properties which in general contribute towards the survival of the plant synthesising them. Specifically they inhibit the growth of certain potentially harmful parasitic plants, possess natural growth regulating properties, and some have important antifungal, antibacterial and antiviral actions.

CHEMICAL STRUCTURE AND PHOTODYNAMIC ACTIVITY

Furocoumarins are formed from coumarin which is produced by the fusion of a pyrone ring with a benzene nucleus. A furan ring may be condensed with a coumarin molecule in twelve different ways producing compounds which can each become the parent of a family of psoralen-like derivatives. However, only those with a linear tricyclic structure resembling psoralen (*Fig. 1*) have important photodynamic, photosensitising and pigmenting actions.

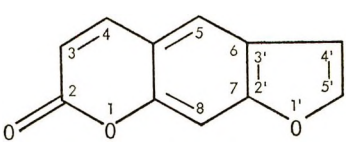


Figure 1. Psoralen.

The term photodynamic action refers to the oxygen dependent lethal or inhibiting changes in living or non-living biological systems which are brought about by the light and the exogenous agent absorbing the energy of light (1). There is a relationship between the molecular structure and the photodynamic activity of psoralens (2) and some correlation between these and the biological activity of such substances (3). The latter depends on the quantum of light absorbed by the psoralen molecule and the emergence of light at another wavelength from a component in intimate contact with sensitive cellular structures (4). Evidently any deviation in the character of light absorption from changing the molecular structure will alter the capacity of the molecule to exhibit its biological effect. The active centres in the psoralen molecule are: the valence bond between carbon atoms 3 and 4; those between carbon atoms 5 and 8; the intact lactone ring; the furan and coumarin fusion at carbon atoms 6 and 7 to give a linear furocoumarin structure; and the unsaturated linkage between carbons 4′ and 5′ (2). In general, any substitution with groups other than methyl diminishes or destroys the photoactivity of the psoralen molecule.

DEVELOPMENT

The ancient Egyptians knew of the pigment-enhancing properties of topically or orally administered extracts of the wild umbelliferous plant *Ammi majus* if followed by sun exposure. Indian medical history reveals an extract from the plant *Psoraea corylifolia* used in a similar manner—for repigmenting what descriptively appears to have been vitiligo. *P. corylifolia* has since been shown to contain psoralen. Extracts from such plants became chemically more sophisticated until pure psoralens were isolated from them. Fahmy and Abu-Shady (5) first reported the isolation and pharmacology of psoralens, the most potent of which proved to be psoralen, 4-methylpsoralen, 5,8-dimethylpsoralen, 5-methoxypsoralen and 8-methoxypsoralen (Methoxsalen; *Fig. 2*). The latter was the most commonly used psoralen derivative until the development of 4,5′,8-trimethylpsoralen (Trisoralen; Trioxsalen, *Fig. 2*). The augmentation of melanin pigmentation of

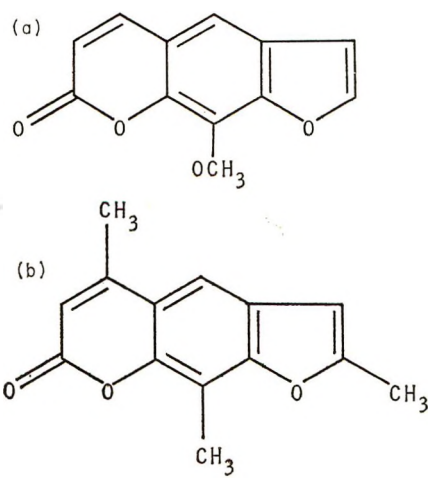


Figure 2. (a) 8-methoxypsoralen (Methoxsalen). (b) 4,5′,8-trimethylpsoralen (Trisoralen; Trioxsalen).

skin induced by psoralens and exposure to natural or artificial U.V.R. (320–400 nm) involves a close interaction between epidermal melanocytes which synthesise melanosomes and the keratinocytes which acquire the melanosomes. The number of functional

melanocytes increases as a result of proliferation and/or activation of melanocytes and increased arborization of melanocyte dendrites is usually also observed. The number of functional melanocytes increases within days and may remain raised for up to 60 days. There also appears to be an increase in the synthesis of melanosomes associated with a more rapid 'turnover' of epidermal keratinocytes, i.e. more epidermal cells containing more pigment than normal. Whether this increase in pigmentation is due solely to direct stimulation of melanogenesis or 'sub-clinical' inflammation also—post-inflammatory hyperpigmentation—is not clear. The latter mechanism is not likely to be operative after topical application of a psoralen since generally speaking this mode of administration provides a more potent photosensitizing stimulus.

METABOLISM

Pure psoralens have been in use for almost 30 years but until recently very little was known of their metabolism after oral ingestion. Trisoralen, Methoxsalen and Psoralen are rapidly metabolized in the liver and excreted in several forms. Trisoralen is hydrogenated at the 4′, 5′ positions and excreted either as a carboxylated or aldehyde derivative of dihydrotrisoralen; hydroxylation at the 3 position may occur leading to excretion of a glucuronide derivative. The metabolism of Psoralen involves glucuronidation, hydroxylation in the 3 position of the lactone ring and conversion to furocoumaric acid. In both man and animals, plasma levels are maximum between two and three hours and photosensitivity greatest 2 to 4 h after an oral dose, whilst 90% is excreted within 8–12 h.

CLINICAL EFFECTS

The increased pigmentation that follows topical or oral psoralens and graded UVR exposure has been studied by many groups. Arnold (6) showed that patients who normally fail to tan and burn easily develop pigmentation within 2 to 6 months of commencing 10 mgs Methoxsalen daily. 20 mgs Methoxsalen daily produces overt pigmentation of the skin within days of commencing treatment (7). Vitiligo patients receiving oral Trisoralen therapy always notice increasing pigmentation of the normal skin (8). Trisoralen has a much greater pigment-inducing tendency than Methoxsalen and this is a major reason for preferring Methoxsalen for the treatment of psoriasis with long wave ultra-violet radiation (U.V.R. or P.U.V.A.) therapy. Imbrie *et al.* (8) have shown that the increased pigmentation and thickening of the stratum corneum produced by psoralens and U.V.R. act as protection against potential sunburn and possibly against skin cancer (10) though the latter, despite being theoretically likely, remains unproven.

There is no doubt that psoralens and graded U.V.R. can lead to repigmentation of vitiliginous skin. The combination of 10 mgs Trisoralen daily followed by exposure to long-wave U.V.R. ('blacklight', U.V.A.) is probably the most potent repigmenting regime but in white and light-skinned individuals deepening pigmentation of normal skin very early in the course of treatment suggests that Methoxsalen is a better choice in all but negroid subjects. The follow-up studies of Kenney (11) have shown that much of the pigment regained during treatment had not receded 8–14 years later.

In the decade 1950–60 psoralens were used in great quantities and largely from a failure to standardize both the psoralen used (particularly topical preparations) and the duration of U.V.R. exposure, burning was unfortunately common. The fact that psoralens were widely thought of as 'suntan pills' also tended to encourage their misuse.

'Non-specific' side effects such as nausea, vomiting, insommia, nervousness, fatigue and drowsiness have been reported from psoralens. However, a double-blind trial by Fitzpatrick (recorded by El Mofty, 1) found a slightly higher incidence of such complaints in the placebo-treated group! El Mofty (1) reported no untoward side effects in patients receiving up to 50 mgs Methoxsalen daily whilst Tucker (12) gave 70 mgs Methoxsalen daily to two patients with no clinical or laboratory evidence of toxicity. Elliott (13) described abnormal cephalic-cholesterol levels in three out of 27 patients receiving Methoxsalen. Since that report virtually all the clinical studies in the literature have outlined results of regular liver and renal function tests and full blood counts, none having proved abnormal whatever the psoralen used. Cloud *et al.* (14) induced cataract formation in mice following intra-peritoneal administration of Methoxsalen in doses 100 times higher than the normal therapeutic range. Cataracts have not been reported in humans but it still remains normal practice to recommend the wearing of protective goggles or sun glasses during treatment.

Caucasian skin has a greater tendency to develop pre-neoplastic keratosis and epitheliomata after many years of sun exposure, as exemplified by the high incidence of such changes in Europeans long resident in Africa or Australia. The theoretical fear that psoralens might accelerate the appearance of epitheliomatous skin changes has fortunately not been realized in practice.

Contra-indications to psoralens are few but it seems reasonable to exclude pregnant women, patients with diseases known to be associated with photosensitivity, e.g. prophyria and lupus erythematosus, and those with known heptaic or renal impairment.

REFERENCES

1 El Mofty, A. M. *In Vitiligo and Psoralens.* 1st Edition, p. 107, Pergamon Press, Oxford.

2 Pathak, M. A., Daniels, F., Hopkins, C. E. and Fitzpatrick, T. B. Ultraviolet carcinogenesis in albino & pigmented mice receiving furocoumarins: psoralen and 8-methoxypsoralen. *Nature* **183** 728 (1959).

3 Pathak, M. A., Fellman, J. H. and Kaufman, K. D. The effect of structural alteration on the erythema activity of furocoumarins: psoralens. *J. Invest. Derm.* **35** 165–183 (1960).

4 Oginky, E. L., Green, G. S., Griffiths, D. G. and Fowlks, W. L. Lethal photosensitization of bacteria with 8-MOP to longwave U.V.R. *J. Bact.* **78** 821 (1959).

5 Fahmy, I. R. and Abu-Shady, H. A. A. Ammini majus Linn: Pharmacognostical study and isolation of crystalline constituent, ammoidin. *Quart. J. Pharm. Pharmacol.* **20** 281–291 (1947).

6 Arnold, H. L. Jr. Effect of methoxsalen on inability to tan. *J. Invest. Derm.* **32** 341–342 (1959).

7 Hoekenga, M. A. Experiences with methoxsalen in the American Tropics. *J. Invest. Derm.* **32** 351–353 (1959).

8 Hopkins, C. E. Psoralen prophylaxis against skin cancer: process of field trials. *J. Invest. Derm.* **32** 383–386 (1959).

9 Imbrie, J. D., Bergeron, L. and Fitzpatrick, T. B. Follow-up study effect of oral methoxsalen (8-methoxypsoralen) on sunburn & suntan. *Arch. Derm.* **82** 617–620 (1960).

11 Kenney, J. A. Jr. Vitiligo treated by psoralens. *Arch. Derm.* **103** 475–480 (1971).

12 Tucker, H. A. Clinical and laboratory tolerance studies in volunteers given oral methoxsalen. *J. Invest. Derm.* **32** 277–80 (1959).

13 Elliot, J. A. Jr. Clinical experience with methoxsalen in the treatment of vitiligo. *J. Invest. Derm.* **32** 311–313 (1959).

14 Cloud, T. M., Hakim, R. and Griffin, A.G. Fertile sensitization of the eye with methoxsalen. Part 2: Chronic effects. *Arch. Ophthal.* **66** 689 (1961).

J. Soc. Cosmet. Chem. **28** 407–412 (1977)

Skin bleaching preparations

S. S. BLEEHEN *Sub-Department of Dermatology, Hallamshire Hospital, University of Sheffield, Sheffield S10 2JF*

Presented at the Joint Symposium with the Pharmaceutical Society of Great Britain "Cosmetic and Pharmacological Aspects of Colour" 9–11 November 1976, at Stratford upon Avon.

Synopsis

A brief review is given of the search for an effective and safe **depigmenting compound** and the **screening** of topically applied chemicals. The possible modes of action of these compounds in producing cutaneous depigmentation are discussed. The results of treatment using several skin bleaching preparations including a new **formulation** of **hydroquinone** and 4-**isopropylcatechol** cream in the therapy of various hypermelanotic disorders in man are stated.

INTRODUCTION

Hyperpigmentation of the skin in man can be the cause of much mental distress and hypermelanotic areas, particularly on the face, can result in a marked cosmetic disability. For a long time, there has been a search for a reliable effective and safe depigmenting compound that when topically applied will bleach away the excess pigment. The compounds that are currently used in the commercially available skin bleaching creams (*Table I*) are variable in their depigmenting effect and frequently irritate the skin, particularly when used in high concentrations. These compounds are occasionally sensitisers and can produce an allergic contact dermatitis. However, in spite of these drawbacks, skin bleaching creams have a considerable world wide sale and vast amounts are purchased, particularly in the United States, Africa and in Asia, mostly over the counter without a doctor's prescription.

Table I. Compounds used in skin bleaching preparations

Hydroquinone
Monobenzyl ether of hydroquinone
Monomethyl ether of hydroquinone
Ammoniated mercury
Ascorbic acid
Peroxides

This paper gives the historical background of the search for an effective depigmenting compound and describes the screening of potent chemicals and their modes of action in producing depigmentation, particularly their melanocytotoxic effect. The clinical

experiences of the author are given in using various skin bleaching creams in the treatment of various disorders of pigmentation in man.

HISTORICAL BACKGROUND—THE SEARCH FOR AN EFFECTIVE DEPIGMENTING PREPARATION

In 1936 Oettel (1) noted that when hydroquinone was fed to black haired cats, their coats turned grey after about 6–8 weeks. Subsequently, in 1940, Oliver, Schwartz and Warren (2) reported a number of cases of depigmentation of the skin occurring among Negro workers in a tannery. This occupational leucoderma was due to an antioxidant, the monobenzyl ether of hydroquinone, which was present in the rubber gloves which these workers wore. The compound was later used in varying concentrations in creams for the treatment of melanin hyperpigmentation (3). It soon was apparent that it was variable in its depigmenting effect and frequently irritated the skin (4). Even more alarming were the frequent reports of confetti-like areas of depigmentation occurring in the treated areas of skin as well as vitiligo-like areas occurring at distant sites which often spread, even after therapy was discontinued. These therapeutic cosmetic disasters are still common and in recent years, there has been an epidemic of monobenzone leucomelanoderma in South Africa affecting perhaps over a thousand cases (5, 6). In addition to the known depigmenting agents—hydroquinone and the monobenzyl ether of hydroquinone, the monomethyl ether of hydroquinone (7) and the monoethyl ether of hydroquinone (8) were found by Brun to be effective depigmenting agents when applied to the pigmented nipples of guinea-pigs.

Studies by Chavin and Schlesinger (9, 10) showed a number of groups of chemical compounds, when injected into black goldfish, had a selective destructive effect on the pigment cells. These workers found several mercaptoamines were potent depigmenting compounds. Two of these, 2-mercaptoethylamine hydrochloride (MEA) and N(2-mercaptoethyl)-dimethylamine hydrochloride (MEDA) were potent depigmenting agents when applied to the skin of black guinea-pigs (11). Both MEA and MEDA, however, are very malodorous and therefore could not be used clinically in man. Other compounds discovered by Chavin and Schlesinger to produce depigmentation in black goldfish were later tested on black guinea-pigs and of these, 4-isopropylcatechol (4-IPC) was found to be the most potent (12). This compound was more effective than known depigmenting compounds, e.g. hydroquinone and the monobenzyl and monomethyl ethers of hydroquinone.

Outbreaks of occupational leucoderma have been reported in Russia by Chumakov, Babanov and Smirnov (13), in Japan by Okumuru and Shirai (14), in Holland by Malten *et al.* (15), in the United States by Kahn (16) and in the UK by Calnan and Cooke (17) among workers in contact with p-tertiary butyl phenol and with p-tertiary butyl catechol (18). However, these substituted phenols cannot be used in the treatment of hypermelanosis in man since, like the monobenzyl ether of hydroquinone, they frequently produce a permanent leucoderma which extends from sites of application to distant areas and also because they often irritate the skin and produce sensitisation. 4-isopropylcatechol has been used in the treatment of hypermelanosis in man (19) but though it is a potent depigmenting agent, it is irritant to the skin and should be used with caution.

Recently, Kligman and Willis (20) have described a new formula for depigmenting human skin using 0·1% tretinoin, 5% hydroquinone and 0·1% dexamethasone as a lotion or cream. These workers found it to be effective in the depigmenting of normal

negro skin as well as in the treatment of various disorders of pigmentation. This formulation of hydroquinone was more potent than standard preparations.

SCREENING COMPOUNDS FOR THEIR DEPIGMENTING EFFECT

Of animal models, the guinea-pig has been found to be most useful for screening topically applied chemical compounds for their depigmenting effect (11, 12, 21). Creams containing concentrations of 1 to 10% of various test chemicals have been applied to the epilated skin of pure black guinea-pigs for five days each week for periods of one month and their depigmenting potency assessed and compared with control areas treated with the cream base only (12). The assessment has been made visually, but also can be measured using a reflectance spectrophotometer.

Of compounds tested using this bioassay method, Bleehen *et al.* (12) found that 4-isopropylcatechol was the most potent. However, when used in concentrations of 3% or more it was irritant to the skin and, like other substituted phenols, it was a sensitizer. So far, no depigmenting compound has been discovered which is reliable, effective and completely safe. All the compounds potent in producing cutaneous depigmentation have certain drawbacks, particularly their irritant effect on the skin.

Other animals with black skins such as pigs have been used to screen compounds but the ultimate test for these compounds is their effect on man. The variation in the response to different species of animals to a drug is often considerable and what may be a potent depigmenting compound for guinea-pigs may not be for man.

MODES OF ACTION OF DEPIGMENTING COMPOUNDS

Exogenous compounds can interfere with the biological processes involved in the production and transfer of pigment granules. The possible modes of action are:-

The compounds may selectively destroy the melanocytes.
They may inhibit the formation of melanosomes and alter their structure.
Inhibit the biosynthesis of tyrosinase.
Inhibit the formation of melanin.
They could interfere with the transfer of melanosomes.
They could have a chemical effect on melanin or enhance the degradation of melanosomes in keratinocytes.

Recent experimental studies have shown that a number of substituted phenols both in vivo (12, 21, 22) and in vitro (23, 24) have a specific melanocytotoxic effect. Hydroquinone produces similar toxic effects on functional melanocytes affecting not only the formation, melanization and degradation of melanosomes, but also producing disruption of membraneous cytoplasmic organelles (25).

Studies on the melanocytotoxic action of the monomethyl ether of hydroquinone and on 4-isopropylcatechol have shown that both compounds have dose dependent lethal effects on cultures of normal guinea-pig melanocytes as shown by Riley (23) and malignant melanocytes by Bleehen (24). It seems likely that these compounds are converted by tyrosinase to form highly toxic oxidation products and electron spin resonance studies indicate that these metabolites are probably free radicals (23). These free radicals initiate a chain reaction of lipid peroxidation resulting in the irreversible damage of the lipoprotein membranes of the melanocyte and producing the death of this cell.

CLINICAL EXPERIENCE OF VARIOUS SKIN BLEACHING PREPARATIONS

Hydroquinone

It is curious that the depigmenting effect of hydroquinone on the skin of man was discovered by chance. The manufacturers of a sunscreen containing this compound found that their preparation was mainly being bought for its effect as a skin bleaching cream (26). Though many workers Spencer (27) and Fitzpatrick *et al.* (28) have found preparations containing 2% to 5% hydroquinone to be effective in producing cutaneous depigmentation, our own experience has been most disappointing. Higher concentrations of hydroquinone, while they are more potent, frequently irritate the skin and if used for a protracted period, can induce an exogenous ochronosis and produce pigmented colloid milium as shown by Findlay *et al.* (5).

A suggested formula using 5% hydroquinone, 0·1% retinoic acid and 0·1% dexamethasone (20) does seem to be promising and is effective in producing depigmentation of human skin. Over the past year, a similar formulation of hydroquinone has been used but instead of dexamethasone, 1% hydrocortisone has been substituted. The formulation used in Sheffield is as follows:-

	% w/v
Hydroquinone	5·00
Hydrocortisone B.P.	1·00
Retinoic Acid	0·10
Butylated hydroxytoluene	0·05
Methylated spirit 74 o.p. }	q.s. ·100 ml
Polyethylene glycol 300 }	47·00

This preparation has been found to be effective in the treatment of various hypermelanotic conditions. A total of sixteen patients have been treated (*Table II*), thirteen of these being female and three male. Most of the female patients had melasma (chloasma) and this was mainly due to taking the contraceptive pill (*Fig. 1*). Two of the male patients had melasma, the other had post-inflammatory hyperpigmentation. The preparation was applied once or twice daily to the hypermelanotic areas and of the twelve patients with melasma, ten were significantly improved (*Fig. 1*), (*Fig. 2*). Two of these patients did not show much change even after 3 months of therapy. One patient with post-inflammatory hyperpigmentation following lichen planus failed to respond and thought that she was made worse. Most of the patients have complained of skin irritation, especially during the early weeks of therapy. In some, erythema and scaling of the treated areas of skin were apparent. A skin bleaching effect was noted within 1 month in a third of the patients but the majority took much longer to respond to treatment and in one case, satisfactory depigmentation occurred only after 3 months of therapy.

Table II. Disorders of pigmentation treated with new formulation of hydroquinone

Disorder	No. of patients treated	No. improved
Melasma	12	10
Post-inflammatory hyperpigmentation	2	1
Vitiligo	2	0

Facing p. 410

Figure 1. Melasma, prior to treatment.

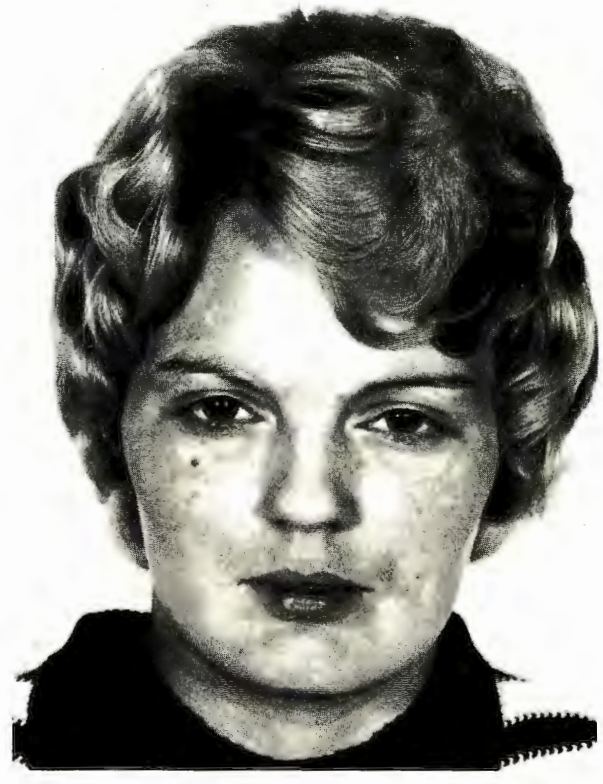


Figure 2. After 2 months therapy using new formulation of hydroquinone. Removal of much of excess pigment in hypermelanotic areas of face.

Monobenzyl ether of hydroquinone

The therapeutic use of the above compound has been the cause of many cosmetic disasters (29) and it should be tried only for bleaching away residual areas of pigmentation in patients with extensive vitiligo (30). My own experience is limited to three patients, all who had extensive vitiligo, and only one who responded well to treatment with 20% monobenzyl ether of hydroquinone ointment. This patient had permanent depigmentation of the treated areas on the face and neck. The others showed no change, probably because they did not persist with treatment. This compound when used in concentrations of 10–20% not infrequently irritates the skin and can produce sensitization.

4-isopropylcatechol

Over the past 6 years, we have used creams containing 1% or 3% 4-isopropylcatechol for the treatment of various hypermelanotic conditions (19). Of the sixty-eight patients treated (*Table III*) two thirds were significantly improved, most of these patients having melasma. Twenty patients had skin irritation due to the compound and four of these patients had an allergic contact dermatitis. One patient, an Asian male, developed confetti-like areas of depigmentation in the treated sides on his face. Though this compound was found to be a most potent and reliable depigmenting agent, it should be used with caution because it frequently irritates the skin and is a potent sensitizer. It could not be used in skin bleaching preparations for sale over the counter because of these side effects.

At the present time, there is no reliable effective and safe skin bleaching cream and the search for this still goes on.

Table III. Disorders of pigmentation treated with 4-isopropylcatechol (19)

Disorder	No. of patients treated	No. improved
Melasma	54	42
Post-inflammatory hyperpigmentation	6	2
Vitiligo	4	2
Pigmented naevi	2	0
Lentigenes	2	0

REFERENCES

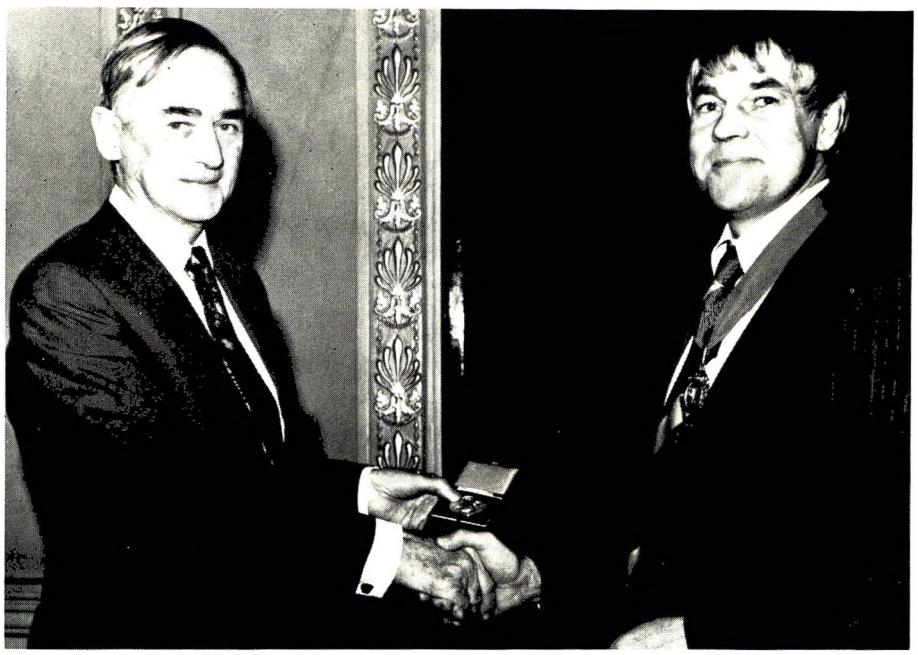
1 Oettel, H. Die Hydrochinonvergiftung. *Archives Exp. Pathol. Pharmacol.* **183** 319 (1936).
2 Oliver, E. A., Schwartz, L. and Warren L. H. Occupational leucoderma. *Arch. Dermatol.* **42** 993 (1940).
3 Lerner, A. B. and Fitzpatrick, T. B. Treatment of melanin hyperpigmentation. *JAMA* **152** 577 (1953).
4 Dorsey, C. S. Dermatitis and pigmentary reactions to monobenzyl ether of hydroquinone. *Arch. Dermatol.* **81** 245 (1960).
5 Findlay, G. H., Morrison, J. G. L. and Simson, I. W. Exogenous ochronosis and pigmented colloid milium from hydroquinone bleaching creams. *Brit. J. Dermatol.* **93** 613 (1975).
6 Dogliotti, M., Caro, I., Hartdegen, R. G. and Whiting, D. A. Leucomelanoderma in blacks. *S. Afr. med. J.* **48** 1555 (1974).
7 Brun, R. Contribution à l'étude de la depigmentation expérimentale. *Bull. Inst. National Genevois* **61** 1 (1961).

8 Brun, R. Effect of ethyl ether of hydroquinone on pigmentation and on the cells of Langerhans. *Dermatologica* **134** 125 (1967).
9 Chavin, W. and Schlesinger, W. Some potent melanin depigmentary agents in the black goldfish. *Naturwissenschaften* **53** 413 (1966).
10 Chavin, W., Schlesinger, W. and Hu, F. *Advances in biology of skin vol. VIII* 421 (Pergamon Press) (1967).
11 Frenk, E., Pathak, M. A., Szabo, G. and Fitzpatrick, T. B. Selective action of mercoptoethylamines on melanocytes in mammalian skin. *Arch. Dermatol.* **97** 465 (1968).
12 Bleehen, S. S., Pathak, M. A., Hori, Y. and Fitzpatrick, T. B. Depigmentation of skin with 4-isopropylcatechol, mercoptoamines and other compounds. *J. Invest. Dermatol.* **50** 103 (1968).
13 Chumakov, N. N., Babanov, G. P. and Smirnov, A. G. Vitiliginoid dermatoses in workers of phenol-formaldehyde resin works. *Bulletin of Dermatology* (Moscow) **4** 3 (1962).
14 Okumura, Y. and Shirai, T. Vitiliginous lesions occurring among workers in a phenol-derivative factory. *Jap. J. Dermatol.* **72** 618 (1962).
15 Malten, K.E., Seutter, E., Hara, I. and Nakajima. Occupational vitiligo due to paratertiary butylphenol and homologues. *Trans. of St John's Hospital Dermatol. Society* **57** 115 (1971).
16 Kahn, G. Depigmentation caused by phenolic detergent germicides. *Arch. Dermatol.* **102** 177 (1970).
17 Calnan, C. D. and Cooke, M. A. Leucoderma in industry. *J. Soc. Occup. Med.* **24** 59 (1974).
18 Gellin, G., Possick, P. A. and Perone, V. B. Depigmentation from 4-tertiary butylcatechol. An experimental study. *J. Invest. Dermatol.* **55** 190 (1970).
19 Bleehen, S. S. Treatment of hypermelanosis with 4-isopropylcatechol. *Brit. J. Dermatol.* **94** 687 (1976).
20 Kligman, A. M. and Willis, I. A new formula for depigmenting human skin. *Arch. Dermatol.* **111** 40 (1975).
21 Riley, P. A. Hydroxyanisole depigmentation: *In-vivo* studies. *J. Path.* **97** 185 (1969).
22 Frenk, E. and Ott, F. Evaluation of the toxicity of the monoethyl ether of hydroquinone for mammalian melanocytes and melanoma cells. *J. Invest. Dermatol.* **56** 287 (1971).
23 Riley, P. A. Mechanism of pigment cell toxicity produced by hydroxyanisole. *J .Path.* **101** 163 (1970).
24 Bleehen, S. S. Selective lethal effects of substituted phenols on cell cultures of malignant melanocytes in Riley, V. *Pigment Cell* **2** 108 (1976) Karger, Basel.
25 Jimbow, K. Obata, H., Pathak, M. A. and Fitzpatrick, T. B. Mechanism of depigmentation by hydroquinone. *J. Invest. Dermatol.* **62** 436 (1974).
26 Arndt, K. A. and Fitzpatrick, T. B. Topical use of hydroquinone for depigmentation. *JAMA* **194** 962 (1965).
27 Spencer, M. C. Topical use of hydroquinone for depigmentation. *JAMA* **194** 962 (1965).
28 Fitzpatrick, T. B. Arndt, K. A., El-Mofty, A. M. and Pathak, M. A. Hydroquinone and psoralens in the therapy of hypermelanosis and vitiligo. *Arch. Dermatol.* **93** 589 (1966).
29 Editorial. Melanocidal compounds. *Trans. of St John's Hospital Dermatol. Society* **56** 181 (1970).
30 Fitzpatrick, T. B., Parrish, J. A. and Pathak, M. A. Phototherapy of vitiligo (idiopathic leucoderma) in Fitzpatrick, T. B. *Sunlight and Man* 783 (1974) (University of Tokyo Press).

J. Soc. Cosmet. Chem. **28** 413 (1977)

Society of Cosmetic Chemists of Great Britain

1977 Medal Lecture Presentation



(Left) Professor Russell J. L. Allen, O.B.E., M.Sc., Ph.D., Group Research Director of the Beecham Group receiving the 1977 Silver Medal of the Society of Cosmetic Chemists of Great Britain from its President, Mr D. F. Williams, C.Chem., MRIC.

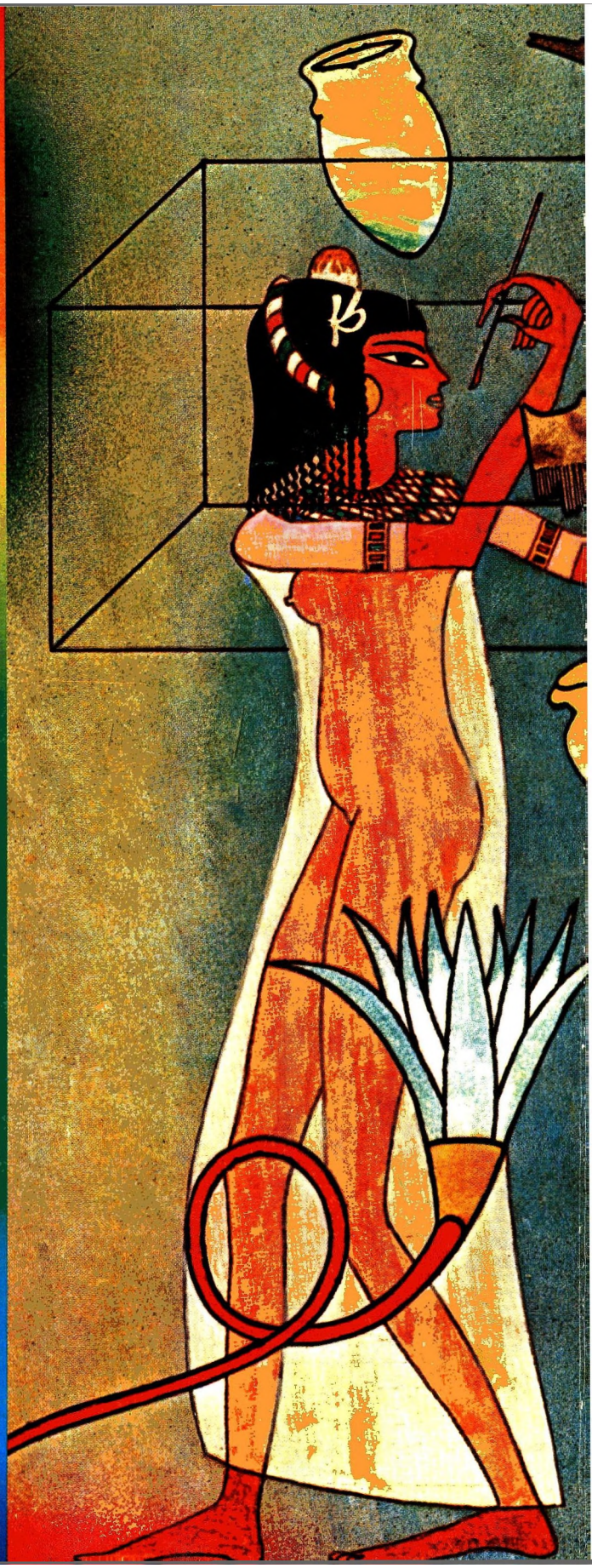
The 1977 Medal Lecture of the Society of Cosmetic Chemists of Great Britain was given to Prof. R. J. L. Allen, O.B.E., M.Sc., Ph.D., on Thursday, 3 March, 1977 at a meeting of the Society held at the Royal Society of Arts.

The medal was presented by the President Mr D. F. Williams, who expressed the thanks of the Society to Professor Allen for his outstanding contributions over many years to the establishment of a scientific basis to the industry.

In his address 'Cosmetics in the future' which followed, Professor Allen reviewed the criticisms which have been made of the industry. He compared the part legislation has played in the food and pharmaceutical industries and underlined the pitfalls which cosmetic legislation would do well to avoid.

Published on behalf of the Society of Cosmetic Chemists of Great Britain by Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL. Printed in Great Britain by Burgess & Son (Abingdon) Ltd, Station Road, Abingdon, Oxfordshire.

Cosmetic Colorants



Sun distributors in Europe

S. Black (Import & Export) Ltd.
Independent Works
Wennington Road
Rainham, Essex, England

Expandia S.A.
13 Ave. de l'Opera
Paris, France

Nordmann, Rassmann & Co.
Kajen 2
Hamburg, West Germany

S.A. Especialidades Quimicas
Via Augusta 137
Barcelona, Spain

Sun Chemical Corporation

Pigments Division